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Review

Role of ERK1/2 and p38 Protein Kinases in Tumors: Biological Insights and Clinical Implications

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Abstract

Significant advancements have been achieved over recent decades in deciphering the molecular mechanisms driving malignant tumor development. Despite this progress, the precise roles of individual genes, their interactions, and the associated signaling pathways involved in tumor proliferation remain insufficiently characterized. Among these pathways, the mitogen-activated protein kinases (MAPKs) extracellular signal-regulated kinase (ERK)1/2 and p38, which regulate essential cellular functions such as growth, differentiation, and apoptosis, have garnered considerable research attention. Building on recent insights into MAPK signaling, we identified components closely linked to ERK1/2 and p38 activity and examined changes in their behavior during tumorigenesis. Furthermore, we developed quantifiable metrics to assess ERK1/2 and p38 activity, including the ERK/p38 ratio, a key indicator of tumor cell proliferative or quiescent states, along with activation levels of these signaling pathways. Our findings underscore the potential of ERK and p38-related gene expression and pathway dynamics as biomarkers for predicting clinical outcomes and informing tailored therapeutic approaches.

Keywords: ERK1 (MAPK3); ERK2 (MAPK1); p38; oncogenesis; gene expression; molecular pathway activation level

1. Introduction

Cancer ranks as the second most common cause of global morbidity, surpassed only by cardiovascular diseases. According to the World Health Organization (WHO), approximately 20 million new cancer cases were reported in 2022, resulting in around 9.7 million deaths, accounting for roughly 17% of all global mortality [1]. Projections indicate that the number of cancer cases could rise to an estimated 35 million by 2050, reflecting a 77% increase from 2022 levels [1]. These alarming trends highlight the critical need for bolstering preventive strategies, advancing early diagnostic techniques, and optimizing therapeutic approaches.

Despite notable progress in cancer diagnosis and treatment, particularly with the advent of targeted therapies, their effectiveness often remains limited [2,3]. Many advanced tumors and entire cancer subtypes frequently exhibit poor responses to these therapies [4–7]. Additionally, drugs deemed ineffective for certain cancer types can occasionally yield substantial therapeutic benefits in specific patient populations [8]. This variability is largely attributed to the complex molecular mechanisms underlying malignant tumor development, which present significant obstacles to the design and success of effective treatments [9,10].

In this context, substantial research has focused on elucidating the molecular pathways that govern cellular processes during oncogenesis. Prominent pathways, including the epidermal growth factor receptor (EGFR) cascade, the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) axis, the rat sarcoma virus protein (RAS)/rapidly accelerated fibrosarcoma kinase (RAF)/MAPK/ERK kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, and the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling network, play pivotal roles in regulating cellular proliferation and growth [11–14]. However, the intricate crosstalk among these pathways creates significant challenges for the development of targeted therapies [15–17]. Dysregulation of these signaling networks is frequently associated with treatment resistance and tumor progression, further complicating therapeutic success [10,11,18–20]. Among these, mitogen-activated protein (MAP) kinases, particularly extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2, encoded by the *MAPK3* and *MAPK1* genes, respectively) and p38 mitogen-activated protein kinases, have emerged as critical targets for advancing cancer research and therapeutic strategies.

The mitogen-activated protein kinase (MAPK) family comprises serine/threonine protein kinases that play a pivotal role in regulating transcription and gene expression. These kinases are integral to a wide range of biological pro-

cesses, including cellular growth, proliferation, differentiation, apoptosis, angiogenesis, and responses to stress stimuli [12,21–23]. MAPK signaling cascades consist of three sequentially activated protein kinases: MAPK kinase kinases (MAP3K or MAPKKK), MAPK kinases (MAPKK or MEK), and MAPKs. These pathways represent highly conserved and ubiquitous regulatory mechanisms across eukaryotic cells, providing coordinated and integrated responses to various external and internal stimuli via receptor-type protein kinases, thereby exerting profound physiological effects at the cellular level [13,21,23].

MAPKs function by phosphorylating target substrates on serine or threonine residues adjacent to proline [23]. Signal specificity and response fidelity are achieved through the action of scaffold, adapter, anchoring, and docking proteins, which organize the MAPK pathway components, enhancing signal transduction efficiency and regulatory diversity [24].

In mammals, fourteen MAPKs have been identified [21], with the most extensively studied being the ERKs, c-Jun N-terminal kinases (JNKs), and p38 isoforms (α, β, γ, and δ) [23]. ERK1/2 is primarily activated by mitogens and is frequently overexpressed in numerous human tumors [25,26]. In contrast, JNKs and p38 kinases, commonly referred to as stress-activated protein kinases (SAPKs), are predominantly activated by stress and genotoxic factors [27–29]. The RAS/RAF/MEK/ERK signaling cascade mediates ERK1/2 activation, a pathway critically involved in tumor cell regulation and strongly linked to the progression and metastasis of various cancers [14,17,25,30]. ERK1/2 kinases serve as a central hub in multiple signaling networks, facilitating tumor cell survival programs, particularly in response to receptor tyrosine kinase inhibitors and conventional chemotherapeutic agents [13,17,31].

Similarly, the p38 kinase governs a diverse array of cellular processes, including proliferation, differentiation, stress responses, apoptosis, migration, and survival [32]. Beyond these functions, p38 also significantly influences the tumor microenvironment and contributes to chemotherapy resistance [33,34].

Given their central roles in cancer biology, MAPKs represent promising targets for anticancer therapies, especially when combined with existing treatment modalities to improve therapeutic outcomes. Identifying patient subgroups that could benefit from MAP kinase inhibitors and elucidating their prognostic and predictive value are critical for optimizing personalized treatment strategies. Moreover, studying MAPK pathways and their interactions with other oncogenic signaling networks is essential for the development of novel therapeutic approaches. Addressing challenges such as drug resistance and metastasis underscores the need for further research on MAPKs, paving the way for advanced and targeted cancer therapies.

2. RAS/RAF/MEK/ERK Signaling Pathway

Among the various MAPKKK/MAPKK/MAPK signaling pathways, the RAF/MEK/ERK cascade is one of the most extensively studied [13]. This evolutionarily conserved pathway plays a critical role in regulating cellular processes such as proliferation, differentiation, the cell cycle, and apoptosis [12,21–23]. Signal transduction within the RAF/MEK/ERK pathway begins with the activation of receptor tyrosine kinases (RTKs), which are commonly triggered by ligands such as growth factors and cytokines [29]. For instance, the activation of the epidermal growth factor receptor (EGFR) leads to autophosphorylation of specific tyrosine residues, which subsequently interact with guanine nucleotide exchange factors (GEFs) [21,35]. These GEFs facilitate the conversion of RAS from its inactive GDP-bound state to the active GTP-bound form (RAS-GTP) at the plasma membrane.

Active RAS-GTP stimulates the dimerization of RAF proteins, forming homo- or heterodimers consisting of A-, B-, or C-RAF. These RAF dimers then phosphorylate and activate MEK1 and MEK2, which, in turn, phosphorylate and activate ERK1 and ERK2 [21,23]. Human ERK1 comprises 379 amino acid residues, while ERK2 consists of 360 residues. Despite slight structural differences, they share 84% sequence identity and exhibit parallel activation and functionality *in vivo* [27,36]. While A-, B-, and C-RAF enzymes specifically target MEK1/2 as their substrates, and MEK1/2 exclusively phosphorylate ERK1/2, ERK1/2 represent critical signaling hubs. These kinases phosphorylate a wide array of cytoplasmic and nuclear proteins, thereby influencing diverse cellular functions (Fig. 1) [37,38].

ERK1/2 also play a complex role in apoptosis, which is particularly relevant in the context of tumor responses to chemotherapy and the activation of intrinsic tumor suppression pathways [17,39]. ERK-mediated apoptosis can occur through intrinsic or extrinsic mechanisms depending on the cell type and the nature of the stimuli. The intrinsic pathway involves mitochondrial cytochrome C release and subsequent caspase-9 activation, whereas the extrinsic pathway triggers apoptosis via caspase-8 activation [40,41]. ERK activity has been linked to the upregulation of pro-apoptotic proteins such as Bax and Bak from the Bcl-2 family, as well as p53, while concurrently downregulating anti-apoptotic proteins, including Bcl-2 and Bcl-xL [17,40,42,43]. These regulatory functions highlight ERK1/2's dual role as mediators of both survival and apoptotic signaling, making them pivotal targets for therapeutic interventions aimed at modulating tumor cell fate.

Mutations in genes encoding receptor tyrosine kinases and the kinases of the RAS/RAF/MEK/ERK pathway frequently result in aberrant activation of ERK1/2, making these mutations one of the most prevalent in cancer. Hyperactivation of the RAS/RAF/MEK/ERK signaling pathway is implicated in 30% to 96% of all tumors [44]. ERK1/2 hyperactivation commonly arises from mutations in RAS,

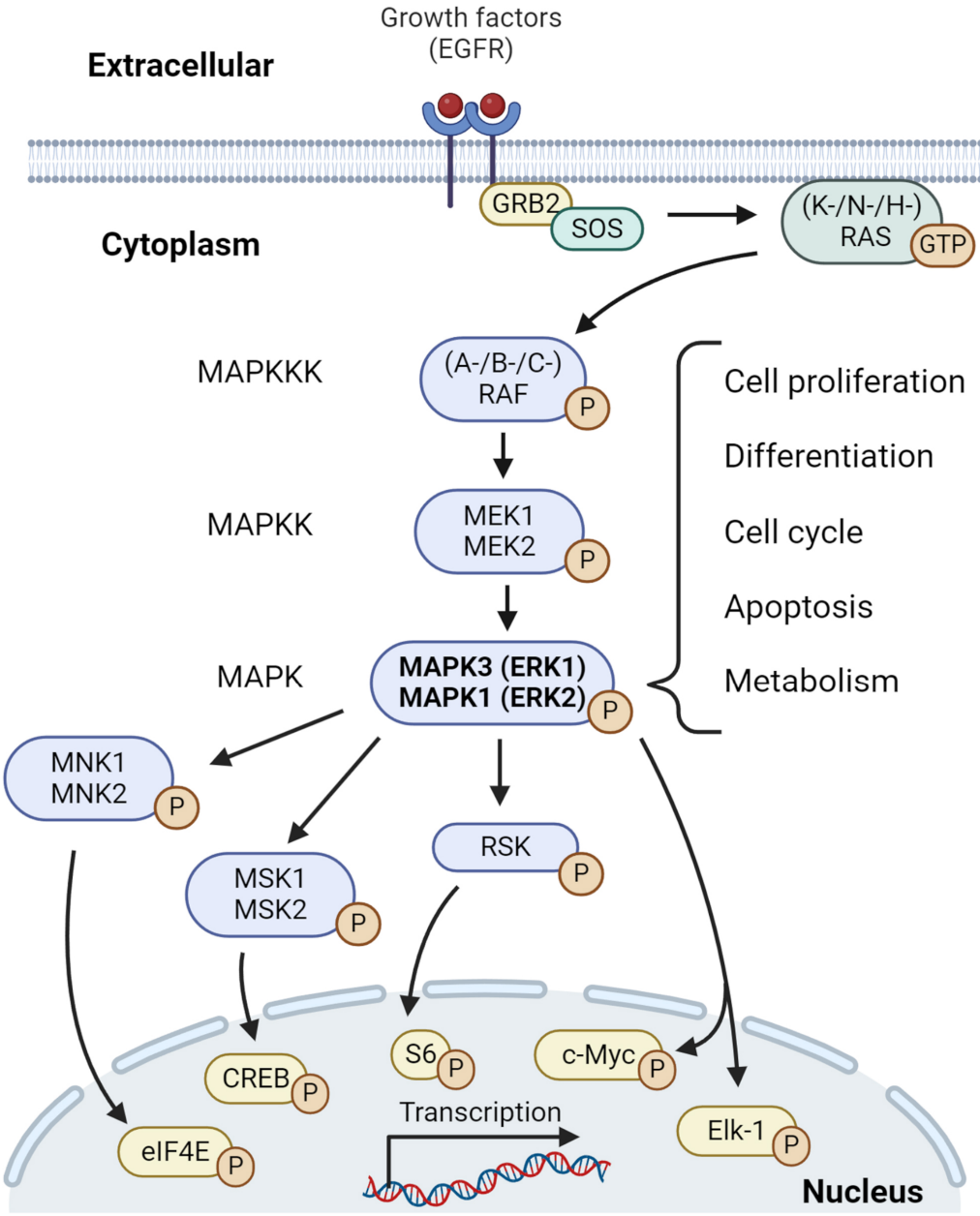

Fig. 1. MAPK/ERK1/2 signaling pathway. Activated ERK regulates a broad spectrum of cellular processes, including transcription, proliferation, differentiation, cell cycle progression, apoptosis, and metabolism, by modulating various downstream effectors. MAPK, mitogen-activated protein kinase; c-Myc, cellular Myc; CREB, cyclic AMP response element-binding protein; EGFR, epidermal growth factor receptor; eIF4E, eukaryotic translation initiation factor 4E; Elk-1, ETS-like protein 1; ERK, extracellular signal-regulated kinase; GRB2, growth factor receptor-bound protein 2; GTP, guanosine triphosphate; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase; MNK, mitogen-activated protein kinase-interacting kinase; MSK, mitogen- and stress-activated protein kinase; P, phosphate; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma; RSK, ribosomal S6 kinase; SOS, son of sevenless. Created with Biorender.com.

RAF, or MEK, which drive uncontrolled activation of this pathway. These mutations have been extensively documented across various cancer types [36]. Additionally, certain long non-coding RNAs, oncogenes, and exosomal signaling contribute to tumor cell proliferation and migration through ERK1/2 activation [27].

Although the ERK1/2 cascade can induce apoptosis, it often promotes cell proliferation and exerts anti-apoptotic effects, underscoring its dual role in oncogenesis. ERK1/2 facilitates its oncogenic effects by aberrantly activating various substrates, including transcription factors such as ETS-like protein 1 (Elk-1), Fos, and members of the Myc fam-

ily. These factors drive proliferative signaling networks that regulate key tumor-associated processes, including cell growth, differentiation, migration, and angiogenesis [27].

One notable downstream substrate of ERK1/2 is mitogen-activated protein kinase-interacting kinase 1/2 (MNK1/2), which influences cell division and survival. Upon activation by ERK1/2, MNK1/2 phosphorylate eukaryotic translation initiation factor 4E (eIF4E) [21,23], promoting the translation of oncogenic proteins [45]. High levels of MNK1/2 have been observed in cancers such as non-small cell lung cancer (NSCLC), melanoma, and acute myeloid leukemia [46].

Another critical substrate of ERK1/2 is ribosomal S6 kinase (RSK). Upon phosphorylation by ERK1/2, RSK is translocated to the nucleus, where it regulates transcription, proliferation, differentiation, cell motility, and survival by modulating specific effectors [47]. Similarly, mitogen- and stress-activated protein kinase 1/2 (MSK1/2), phosphorylated by ERK1/2, activates transcription factors and nuclear proteins, including nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB), cyclic AMP response element-binding protein (CREB), activating transcription factor 1 (ATF1), high-mobility group nucleosome-binding domain 1H (HMGN1), and histone H3. These proteins govern processes such as cell division and the cellular response to stress [48]. Together, these downstream effectors highlight the central role of ERK1/2 in oncogenesis and its potential as a therapeutic target in cancer.

The E-twenty-six (ETS) family of transcription factors includes key substrates of ERK1/2 [27]. Elevated ETS activity drives processes such as epithelial-mesenchymal transition (EMT), DNA damage, genomic instability, and angiogenesis [49]. Elk-1, another ERK1/2 effector, translocates to the nucleus upon activation, where it induces the transcription of genes involved in tumorigenesis across various cancer types [22,50]. In breast cancer, ERK activation upregulates Elk-1 level, promoting Snail phosphorylation and E-cadherin suppression, which facilitates tumor metastasis [51]. Similarly, in colorectal cancer, phosphorylated Elk-1 (p-Elk-1) enhances carcinoembryonic antigen transcription, leading to increased adhesion, migration, and invasion [52].

Another critical proto-oncogene regulated by this pathway is cellular Myc (c-Myc) [53], which is amplified in roughly 40% of human tumors [54]. ERK1/2 phosphorylate Myc at serine residue 62, preventing its degradation, stabilizing the protein, and amplifying its regulatory effects on gene families [53]. This stabilization drives cellular proliferation, metabolic reprogramming, and metastatic potential [55]. Likewise, cellular Fos (c-Fos) can be phosphorylated directly by ERK1/2 or indirectly through RSK activation. This process promotes tumor progression and metastasis. For instance, in squamous cell carcinoma of the head and neck, elevated c-Fos level correlates with increased cancer stem cell marker expression and EMT facilitation [56].

ERK1/2 are also essential mediators of tumor cell stress responses [17]. In epithelial and hematological malignancies, exposure to anticancer therapies activates an ERK1/2-driven emergency survival program [57–59]. This activation occurs in response to receptor tyrosine kinase inhibitors [60] and standard chemotherapies such as cisplatin, carboplatin, paclitaxel, docetaxel, 5-fluorouracil, and doxorubicin [31]. During acute stress, ERK1/2 signaling enables tumor cells to evade apoptosis by activating protective molecular pathways [61,62]. Concurrently, it induces the upregulation of pro-proliferative surface receptors, enabling cells to respond to even minor local increases in growth factor concentrations. This reactivation of proliferative programs ensures tumor survival and regrowth after transient disruptions [61,62].

The ERK1/2 signaling network thus plays a dual role, promoting tumor progression and metastasis while exhibiting pro-apoptotic effects depending on the cellular context. The extensive range of ERK1/2 substrates likely accounts for the low mutation frequency of these kinases and underscores their potential as therapeutic targets. Combining ERK1/2 inhibitors with other anticancer agents represents a promising strategy to enhance treatment efficacy [63].

3. Molecular Mechanisms and Functions of p38 Kinase

MAPK p38 is a group of serine/threonine-specific protein kinases critical for signaling pathways that regulate numerous biological processes, including inflammation, cellular stress, apoptosis, and other key cellular responses [32,34,64,65]. The p38 family consists of four isoforms: p38α (MAPK14), p38β (MAPK11), p38γ (MAPK12), and p38δ (MAPK13) [64,66]. While p38α and p38β are ubiquitously expressed and share approximately 75% sequence identity, p38γ and p38δ exhibit tissue-specific expression patterns, sharing 70% identity with each other and 62% and 61% identity with p38α, respectively [67,68]. p38γ is predominantly expressed in skeletal muscle, whereas p38δ is primarily localized in tissues such as the pancreas, kidney, small intestine, testes, and lung [68].

Functionally, p38 isoforms contribute to distinct cellular and physiological processes. p38α and p38β are essential for heart development, mitotic entry, and regulatory T-cell induction and formation. Conversely, p38γ and p38δ play roles in coordinating tissue regeneration and regulating immune responses [33].

The activation of p38 kinase occurs in response to a variety of stress stimuli, including cytokines, genotoxic agents, osmotic stress, reactive oxygen species (ROS), and both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [28,69]. These stimuli activate upstream kinases, such as mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase (MEKK) or mixed-lineage kinase (MLK), which phosphorylate and activate mitogen-

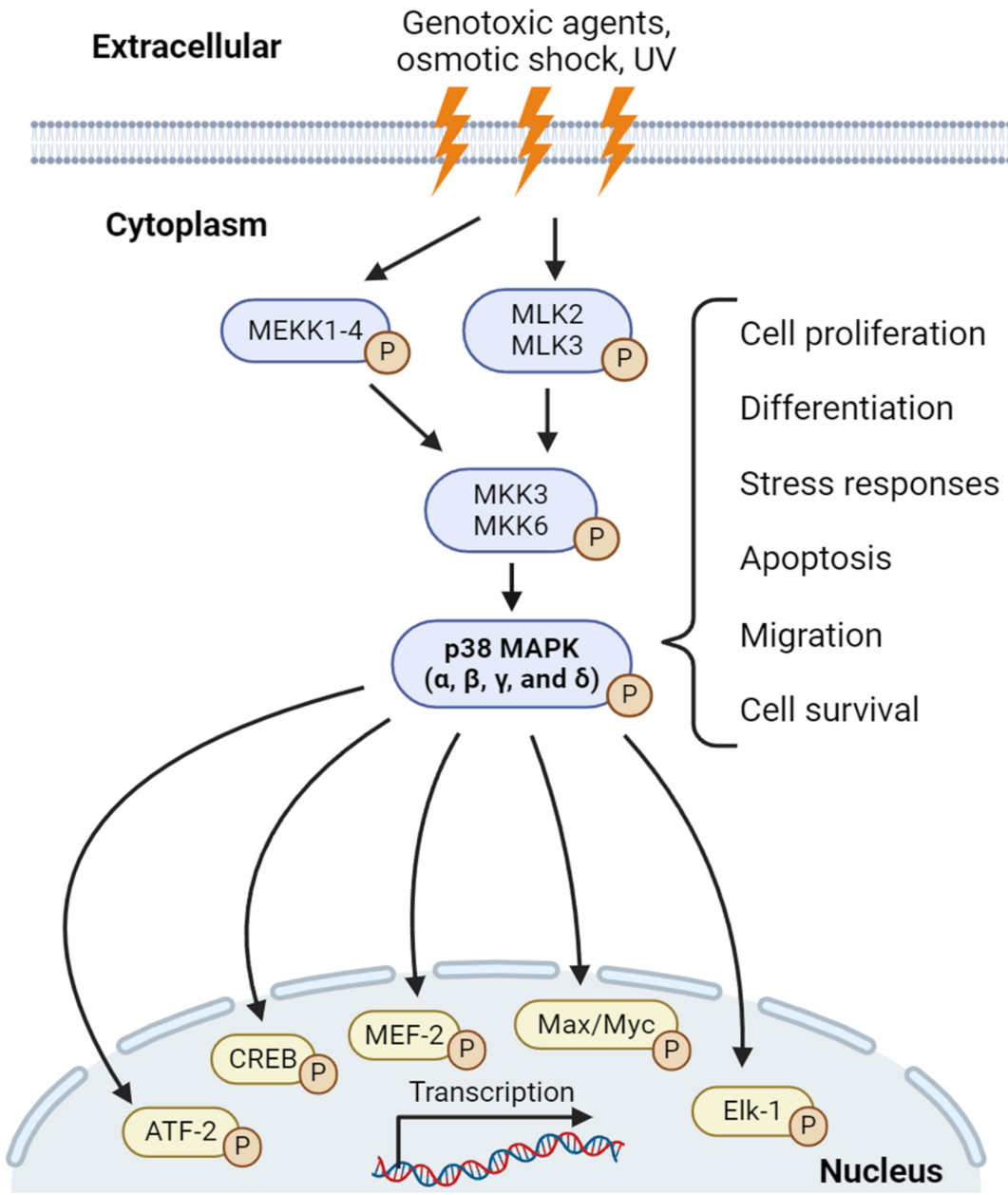

Fig. 2. p38 MAPK signaling pathway. Activated p38 kinase regulates a wide range of cellular processes, including cell proliferation, differentiation, stress responses, apoptosis, migration, and survival. ATF-2, activating transcription factor 2; MEF-2, myocyte enhancer factor 2; MKK, mitogen-activated protein kinase kinase; MLK, mixed-lineage kinase; MEKK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase; UV, ultraviolet. Created with Biorender.com.

activated protein kinase kinase 3/6 (MKK3/6). Subsequently, MKK3/6 phosphorylates and activates p38 kinase. Once activated, p38 regulates a diverse array of cellular functions, such as proliferation, differentiation, apoptosis, migration, stress responses, and survival [28,32,34,64].

Activated p38 kinase influences numerous downstream targets, including heat shock proteins, kinases, and transcription factors. Examples of such transcription factors include ATF-2, STAT1, the Max/Myc complex, myocyte enhancer factor 2 (MEF-2), Elk-1, and CREB, which collectively regulate the expression of genes involved in inflammation and apoptosis [66,70] (Fig. 2). This wide range of regulatory roles underscores the importance of p38 in maintaining cellular homeostasis and responding to environmental and intracellular stress.

p38 kinase plays a multifaceted role in regulating cellular processes, including proliferation, differentiation, stress responses, apoptosis, migration, and survival, as well as cell cycle progression at key checkpoints such as G1/S and G2/M [65,66,71]. In response to DNA damage or cellular stress, p38 kinase acts as a safeguard to maintain homeostasis. For instance, at the G1/S checkpoint, it promotes

cyclin D1 degradation to prevent S-phase entry, while at the G2/M checkpoint, it activates p53, leading to cell cycle arrest [65]. However, p38 can also exhibit pro-proliferative activity under certain conditions, such as in hematopoietic cells, with the outcome depending on the duration and intensity of the activating stimuli [28,72].

Depending on the context, p38 can act as either a tumor suppressor or a tumor promoter [28]. Its tumor-suppressive roles include inducing cell cycle arrest and promoting apoptosis in response to stress or ROS. For example, p38 mediates apoptosis via ROS activation [66,73] and supports tumor suppression by promoting cellular senescence and halting proliferation [28]. Paradoxically, p38 activation by ROS in hepatocellular carcinoma cells can also protect against caspase-independent cell death caused by lysosomal damage, demonstrating its context-dependent duality [74].

In contrast, p38 also supports tumor progression by regulating the production of pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukin-6 (IL-6), IL-1, cyclooxygenase 2 (COX-2), and IL-17, which contribute to tumor growth and the tumor microenvironment [75]. Evidence from *in vivo* models of lung, liver, breast, colon, and skin cancers indicates that p38 hyperactivation can both suppress and promote carcinogenesis depending on the specific cellular and environmental context [75].

- p38 kinase promotes the emergence and maintenance of cancer stem cells, which are pivotal for tumor initiation, recurrence, progression, and metastasis. It activates EMT-related proteins like forkhead box C2 (FOXC2) and zinc finger E-box-binding homeobox 1 (ZEB1), facilitating metastatic processes [33]. Furthermore, chemotherapy-induced transcription factors can enhance p38 activation, amplifying its role in cancer stem cell formation and survival [71].
- p38 contributes to resistance mechanisms by maintaining cancer stem cells in a quiescent state through cyclin D1 inhibition, reducing their vulnerability to chemotherapeutic agents targeting rapidly dividing cells [33,66]. Additionally, p38 enhances resistance by upregulating aldehyde dehydrogenases (ALDH), which detoxify chemotherapy metabolites, and activating ABC transporters directly or through the WNT/β-catenin pathway. These transporters expel chemotherapeutic agents, thereby diminishing their effectiveness [76–81]. p38-mediated G2 checkpoint activation further enables DNA repair, promoting cell survival post-treatment with DNA-damaging agents [33].
- p38α induces the expression of metalloproteinases, aiding extracellular matrix degradation and remodeling, which are crucial for cancer cell invasion and metastasis [71]. Additionally, p38α promotes the production of vascular endothelial growth factor A (VEGF-A), driving angiogenesis and enhancing tumor survival under hypoxic conditions [71].

Beyond cancer, p38 kinase has a well-documented role in neurodegeneration, contributing to diseases like Alzheimer's, Parkinson's, and Huntington's. It exacerbates these conditions through neurotoxic effects and the regulation of pro-inflammatory mechanisms [70].

The dichotomous role of p38 kinase—as both a tumor suppressor and promoter—highlights its complexity as a therapeutic target. Strategies combining p38 inhibitors with other anticancer agents may offer a promising approach to overcoming chemotherapy resistance and mitigating its pro-tumorigenic effects while preserving its anti-oncogenic functions. Further research is crucial to delineate the context-specific roles of p38 and develop effective targeted therapies.

p38 kinase plays a significant role in cancer development by facilitating interactions between tumor cells and components of the tumor microenvironment (TME) [34,71,82]. In cancer, normal cells are recruited to the tumor site to support malignant growth. Among the first responders are fibroblasts, which constitute a major component of the stroma in many solid tumors [83]. Although fibroblasts typically act as tumor suppressors, they can be reprogrammed into cancer-associated fibroblasts (CAFs) through direct interactions with cancer cells [84]. This transformation is critical for oncogenesis, as it drives extensive tissue remodeling and establishes a dynamic network of cytokines, chemokines, growth factors, and matrix remodeling enzymes. These changes alter the tumor's physical and chemical properties, enhancing its ability to grow and invade [71,82,84].

The tumor-promoting microenvironment is further amplified through the recruitment of other cell types, including adipocytes and pericytes, alongside the formation of vascular and lymphatic systems supporting the tumor [83,84]. In a lung cancer model, p38 MAPK was shown to play a pivotal role in transforming the normally suppressive stroma into a pro-tumorigenic environment [85]. This transformation involves p38-induced upregulation of *Has2* expression in fibroblasts, leading to the synthesis and accumulation of hyaluronan. Hyaluronan, in turn, interacts with the CD44 receptor on cancer cells, promoting their growth [85].

Additionally, p38 kinase contributes to tumor progression by modulating interactions with tumor-associated macrophages and regulatory T-cells in the TME, which further enhance tumor growth and metastasis [71].

Similar to ERK1/2 kinases, the role of p38 is highly context-dependent and can be either pro-tumorigenic or anti-tumorigenic. While its involvement in stromal reprogramming and immune cell interactions often supports tumor progression, p38 can also suppress tumors under certain conditions, such as by inducing apoptosis or promoting senescence. This duality underscores the complexity of its function and highlights the need for further research.

Understanding the context-specific roles of p38 kinase in oncogenesis is critical for advancing targeted therapies. Developing p38 inhibitors or combination therapies that selectively modulate its tumor-promoting activities while preserving its anti-tumorigenic effects could significantly enhance cancer treatment efficacy.

4. ERK/p38 Ratio

In addition to their well-established roles in tumor development and progression, ERK and p38 kinases also interact in the regulation of dormant cancer cells [33,86–88]. Dormant cancer cells are a subpopulation of tumor cells that can persist in a latent state within target organs for prolonged periods, awaiting favorable conditions to resume growth and initiate tumor progression [89]. Clinically, these cells remain asymptomatic and undetectable, but under conducive conditions, they can re-enter the cell cycle, proliferate, and cause disease recurrence [88–90]. Dormant cells are often resistant to chemotherapy, radiotherapy, and immunotherapy, making them a significant challenge in cancer treatment. Recurrences that occur years, sometimes even decades, after remission are frequently attributed to the presence of these dormant cells [86,91].

One key mechanism inducing dormancy involves alterations in cellular responses to growth factors and adhesion signals [88,92]. Growth factors such as epidermal growth factor (EGF), transforming growth factor beta (TGF-β), and insulin-like growth factor (IGF) are critical for tumor cell proliferation and survival, while adhesion signals derived from fibronectin and integrins facilitate attachment to the extracellular matrix (ECM) and surrounding cells. When these interactions are disrupted, the tumor cells' ability to interpret environmental signals diminishes, leading to the suppression of the RAS/RAF/MEK/ERK pathway and arrest in the G0/G1 phase of the cell cycle [25,92,93].

For instance, fibronectin-mediated signals involving the urokinase plasminogen activator receptor (uPAR) and $\alpha5\beta1$-integrins promote tumor progression by recruiting focal adhesion kinase (FAK) and EGFR, which transmit mitogenic signals via the RAS/RAF/MEK/ERK pathway while inhibiting p38 MAPK signaling [88,94]. Experimental inhibition of uPAR, $\beta1$-integrins, FAK, and EGFR has been shown to induce dormancy and suppress tumor growth *in vivo*. Western blot analyses using anti-ERK and anti-phospho-ERK antibodies have confirmed reduced ERK protein and phosphorylation levels during dormancy [95].

The interplay between ERK and p38 is crucial for the induction of tumor dormancy. Loss of growth factor receptor signaling triggers activation of p38 MAPK while suppressing ERK signaling. This shift favors stress-induced signaling pathways over proliferative pathways, leading to cell cycle arrest and dormancy. Specifically, p38 activation and ERK suppression mediate cellular responses to environmental stress and growth arrest, enabling tumor cells to enter a quiescent state. This delicate balance underscores the opposing roles of ERK (proliferation-promoting) and p38 (stress-activated dormancy-inducing) signaling in determining cell fate.

Understanding the molecular dynamics of ERK and p38 in tumor dormancy has significant therapeutic implications. Targeting the pathways involved in maintaining dormancy, such as stress signaling through p38 and growth factor receptor signaling through ERK, could provide a means to either eliminate dormant cells or prevent their reactivation. Strategies that modulate this balance—such as inhibiting uPAR, $\beta1$-integrins, or EGFR—may offer potential avenues to suppress tumor growth and recurrence by inducing or maintaining dormancy in residual cancer cells.

Another key mechanism driving cancer cell dormancy is the tumor's inability to stimulate angiogenesis, leading to hypoxia. Under hypoxic conditions, some tumor cells undergo cell death, while others adapt and enter a dormant state [86,88]. This process is governed by the balance between pro-angiogenic factors, such as VEGF and platelet-derived growth factor (PDGF), and anti-angiogenic factors, including thrombospondin, angiostatin, and endostatin, which collectively regulate the switch between proliferative and dormant states [96].

A third mechanism involves suppression of tumor cell proliferation by the immune system. Specifically, cytotoxic CD8+ T cells mediate an immune response that halts tumor growth and induces dormancy [92].

The balance between ERK and p38 kinase activity plays a critical role in the regulation of dormancy. Metastatic tumors typically exhibit high levels of ERK and low levels of p38, enabling sustained proliferative signaling. In contrast, dormant cancer cells maintain low ERK activity and high p38 activity, promoting stress-induced quiescence [33,86,87]. Experimental inactivation of p38α and/or p38β in dormant cells has been shown to reactivate ERK-driven proliferative pathways, facilitating their exit from dormancy and resumption of growth [33,86,87]. Consequently, the ERK/p38 ratio serves as a reliable biomarker for distinguishing dormant cells, with a high ratio associated with proliferation and a low ratio indicative of dormancy (Fig. 3) [86,87,92].

Current therapeutic approaches targeting dormant cancer cells fall into three main strategies:

1. Reactivating Dormant Cells. By inducing dormant cells to re-enter the cell cycle, they become susceptible to conventional anti-proliferative therapies such as chemotherapy and radiotherapy [88,97].

2. Prolonging Dormancy. Therapies designed to maintain or extend the dormant state aim to delay recurrence by suppressing proliferative signaling and enhancing stress pathways like p38 MAPK [88,97].

3. Eliminating Dormant Cells. Emerging targeted therapies focus on selectively eradicating dormant can-

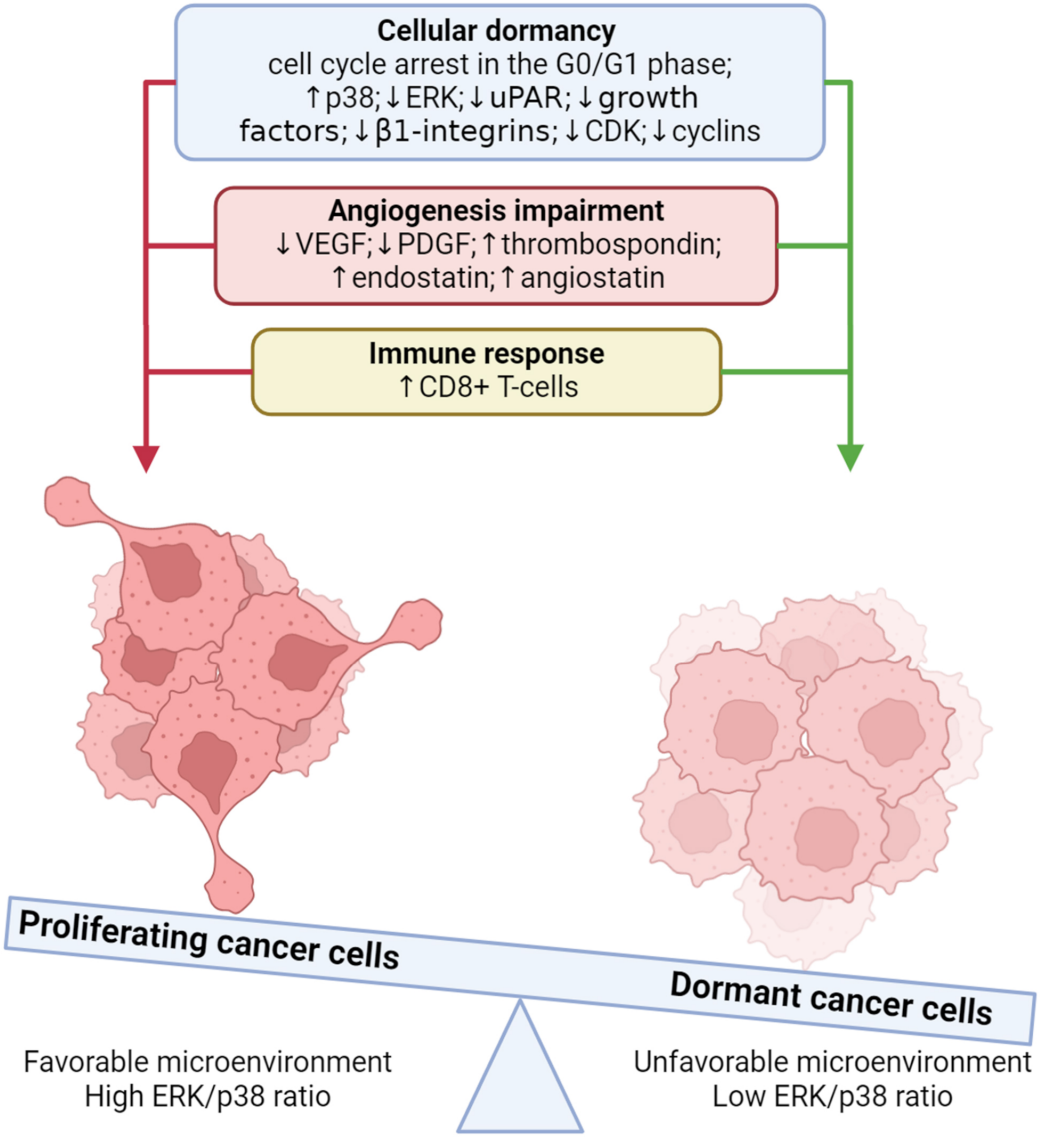

Fig. 3. The effect of ERK/p38 ratio on tumor status. In a favorable microenvironment characterized by a high ERK/p38 ratio, cancer cells are more prone to metastasis and proliferation. Conversely, in an unfavorable microenvironment with a low ERK/p38 ratio, cancer cells are driven into a dormant state, suppressing their proliferation and metastatic potential. Green arrows represent conditions or signals that support dormancy, such as stress-induced activation of p38 and inhibition of proliferative pathways, while red arrows indicate factors promoting the proliferative state, including enhanced ERK signaling and favorable adhesion or growth factor interactions. ↑ and ↓ arrows indicate an increase or decrease in the levels of factors affecting the cancer cell state, respectively. CDK, cyclin-dependent kinase; PDGF, platelet-derived growth factor; uPAR, urokinase plasminogen activator receptor; VEGF, vascular endothelial growth factor. Created with Biorender.com.

cer cells. For example, inhibition of key components of integrin-mediated signaling pathways, including uPAR, β1-integrins, and ERK1/2, has shown promise in preventing dormant cells from reactivating and entering the proliferative phase [94]. These strategies aim to minimize recurrence and improve long-term outcomes by addressing the resilient dormant cell population.

Therapeutic manipulation of the ERK/p38 ratio, angiogenesis regulators, and immune-mediated suppression presents a promising avenue for addressing the challenges posed by dormant cancer cells. Continued research into these mechanisms will likely refine existing approaches and lead to innovative treatments tailored to both primary and metastatic cancers.

The application of p38 kinase inhibitors must be carefully considered, as these inhibitors could unintentionally promote cancer progression and metastasis under certain conditions [28]. The multifaceted roles of p38 proteins in various biological processes also raise concerns about potential off-target effects and adverse outcomes. To effec-

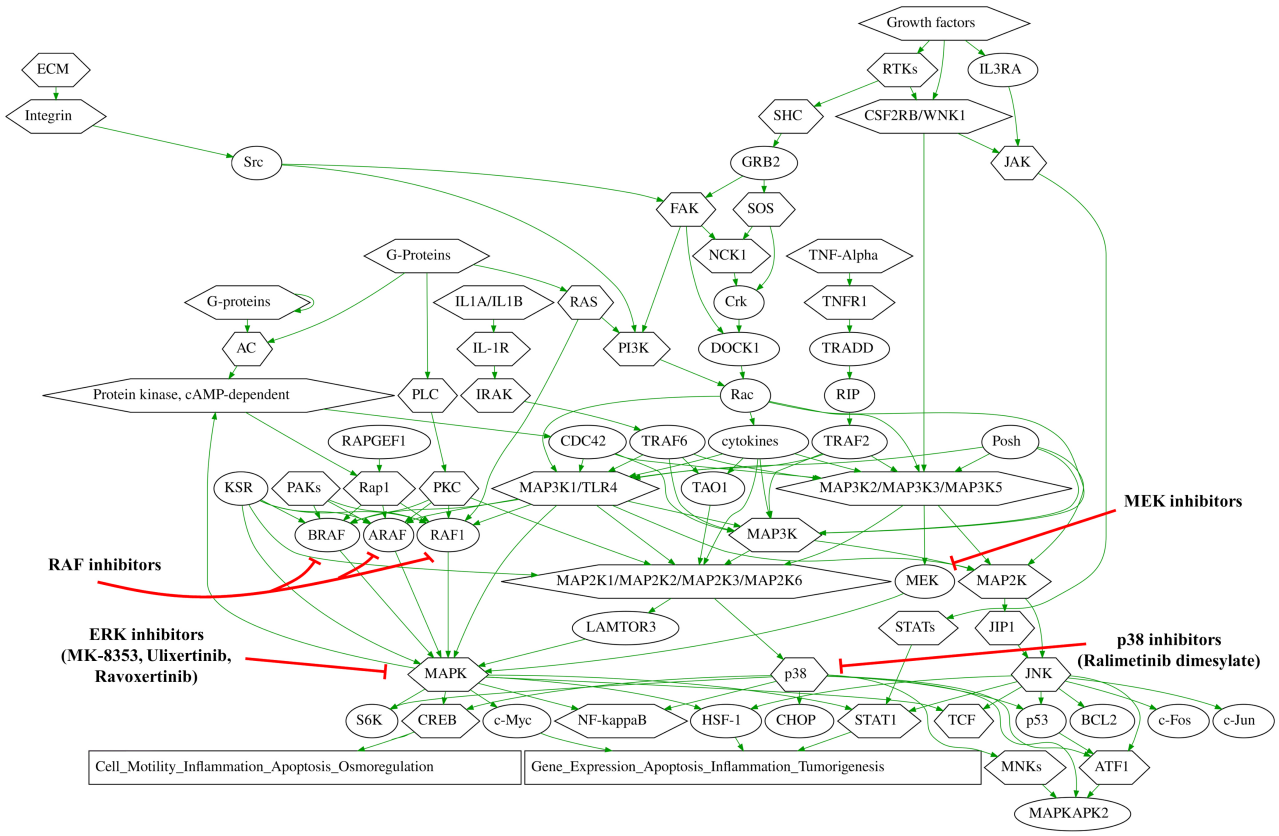

Fig. 4. General scheme of signal transduction involving MAP kinases. Shared intermediates, including upstream kinases, downstream transcription factors, and scaffold proteins, play pivotal roles in modulating divergent outcomes depending on the cellular context and the interaction with other signaling cascades. These intermediates integrate signals from both ERK1/2 and p38 pathways, influencing cell fate decisions such as proliferation, survival, dormancy, or apoptosis. Individual nodes within these pathways represent genes or clusters of genes, each contributing to the dynamic regulation of cellular responses. Green arrows illustrate the activation of specific nodes, highlighting the flow of signaling and the promotion of downstream effects. Red lines indicate the inhibitory action of targeted molecules, reflecting points where specific inhibitors disrupt signaling to modulate therapeutic outcomes. Understanding how these shared components regulate context-specific outcomes provides valuable insights for designing interventions that selectively modulate ERK1/2 or p38 activity. This approach enables precise targeting of cancer cell behavior while reducing the risk of off-target effects and adverse outcomes. Created with Biorender.com.

tively harness p38 as a therapeutic target, it is critical to delineate the specific tissues and cancer types where p38 activation either enhances or suppresses cancer stem cell populations. The diverse roles of p38 within different signaling pathways can yield conflicting outcomes, adding complexity to its therapeutic targeting [28].

Similarly, the therapeutic inhibition of ERK1/2 requires careful evaluation to determine which tumor types are most likely to benefit. Due to the intricate network of shared components between ERK1/2 and p38 pathways, their interplay must also be considered. As shown in Fig. 4, the MAPK pathway includes shared nodes targeted by inhibitors, reflecting the potential for these pathways to act synergistically or antagonistically in promoting cancer cell survival, invasion, and dissemination, particularly in response to environmental stressors such as inflammation [17,28].

Under specific conditions, ERK1/2 and p38 pathways may work in tandem to support tumor progression. For example, their combined activities can facilitate cancer cell adaptability and survival in the dynamic tumor microenvironment. Conversely, these pathways can exhibit opposing effects depending on the cellular context, with regulatory mechanisms and shared components modulating the ERK/p38 ratio [86,94,98]. This interplay significantly influences tumor aggressiveness and plasticity, enabling cancer cells to adapt to diverse environmental stimuli and stress conditions.

Targeting the ERK1/2 and p38 pathways requires a nuanced approach that accounts for their dynamic interactions and context-dependent effects. Strategies aimed at disrupting shared components or modulating the ERK/p38 ratio could yield effective therapeutic outcomes while minimizing adverse effects. Future research should focus on map-

ping the specific roles of these pathways across different tumor types and microenvironments to identify optimal therapeutic targets and refine the use of MAPK inhibitors.

The shared components within the ERK1/2 and p38 pathways highlight the therapeutic challenges posed by their complex crosstalk, which can substantially influence chemoresistance (Fig. 4). Many critical nodes, including upstream kinases, downstream transcription factors, and scaffold proteins, are integral to both pathways, complicating selective targeting without inducing compensatory activation or unintended side effects.

For example, targeted inhibitors of BRAF and MEK proteins are widely used in treating cancers harboring oncogenic mutations in the RAS/RAF/MEK/ERK pathway [12,16,18,30]. While these therapies have shown clinical efficacy, resistance often develops, with one key mechanism being the reactivation of ERK signaling [31,36]. Resistance to RAF, MEK, and ERK inhibitors arises through multiple, intricate mechanisms, including:

1. Mutations in Target Proteins: Secondary mutations in RAF, MEK, or ERK proteins can diminish the efficacy of targeted inhibitors, allowing cancer cells to bypass the blockade.

2. Crosstalk Between Pathways: Interactions between the ERK1/2, p38, and PI3K signaling cascades create alternative proliferative and survival pathways, undermining the effects of MAPK inhibitors.

3. Disruptions in Feedback Regulation: Inhibition of ERK disrupts negative feedback mechanisms within the MAPK cascade, often leading to the upregulation of receptor tyrosine kinases (RTKs) and enhanced RAS activity, which reactivates the pathway.

4. Genetic Alterations: Additional resistance mechanisms include:

- RAS Mutations: Activate upstream components, bypassing RAF/MEK inhibitors.

- *BRAF* Amplifications: Increase signaling output from the RAF node.

- Constitutive Dimerizing Variants of BRAF (e.g., BRAF V600E): Enable RAF activation independent of RAS.

- MEK Mutations: Confer resistance to MEK inhibitors by altering the drug-binding site [99,100].

Effectively targeting the ERK1/2 and p38 pathways requires strategies that account for these resistance mechanisms and the compensatory dynamics within the signaling network. Combination therapies, such as co-targeting MAPK pathways and PI3K/AKT signaling or inhibiting RTKs alongside MAPK inhibitors, may prevent or overcome resistance. Additionally, identifying and disrupting the specific feedback loops or mutations driving resistance in individual tumors can improve the precision and durability of MAPK-targeted therapies.

5. The Efficacy of ERK1/2 and p38 Inhibitors in Monotherapy and Combination Therapy With Other Anticancer Agents

Clinical trials registered on ClinicalTrials.gov were analyzed to evaluate eight ERK1/2 inhibitors—Selumetinib (AZD6244), MK-8353 (SCH900353), AZD0364 (ATG-017), CC-90003, KO-947, Temuterkib (LY3214996), Ulixertinib (BVD-523, VRT752271), and Ravoxertinib (GDC-0994)—as well as ten p38 inhibitors: Doramapimod (BIRB 796), Ralimetinib dimesylate (LY2228820), VX-702, PH-797804, Neflamapimod (VX-745), TAK-715, Losmapimod (GW856553X), Dilmapimod (SB-681323), BMS-582949, and Pexmetinib (ARRY-614).

Only clinical trials with published results on ClinicalTrials.gov were included in this analysis. For ERK1/2 inhibitors, data were available for four drugs:

- Selumetinib (AZD6244): 60 trials
- MK-8353 (SCH900353): 2 trials
- Ulixertinib (BVD-523, VRT752271): 6 trials
- Ravoxertinib (GDC-0994): 2 trials

For p38 inhibitors, published data were available for a single agent: Ralimetinib dimesylate (LY2228820) from five trials. Notably, results for other p38 inhibitors pertained to non-oncological diseases, such as Crohn's disease, rheumatoid arthritis, atherosclerosis, chronic obstructive pulmonary disease, Alzheimer's disease, and dementia.

Efficacy Criteria

For each drug, efficacy was assessed using the following criteria:

- Overall Survival (OS)
- Objective Response Rate (ORR)
- Progression-Free Survival (PFS)
- When PFS data were unavailable, Time to Progression (TTP) or Time to Event (TTE) were used as substitutes.

Additional metrics included:

- Best Overall Response (BOR)
- Presence of Efficacy-Related Mutations

Survival outcomes in these studies were determined using standard statistical tools, including the Kaplan-Meier method, log-rank tests, and Cox regression models, to evaluate differences between treatment groups. Details of these outcomes, along with efficacy-related mutation data, were documented and are available in **Supplementary Tables 1,2**.

This analysis highlights the importance of linking survival metrics to drug-specific efficacy, providing a framework for refining targeted cancer therapies based on their impact on clinically relevant outcomes.

5.1 ERK1/2 and p38 Inhibitors in Monotherapy

In most clinical trials analyzed, only Objective Response Rate (ORR) values were reported, facilitating a direct comparison of drug efficacy based on this metric (**Supplementary Table 1**). A threshold ORR of 30% was

established to define conditional efficacy, aligning with the criteria for regulatory approval of single-agent therapies [101].

p38 Inhibitor: Ralimetinib Dimesylate (LY2228820)

In a phase I clinical trial evaluating Ralimetinib as a monotherapy for advanced solid tumors, the drug demonstrated no efficacy, with an ORR of 0%. The best outcome observed was disease stabilization in 20% of patients [102].

ERK1/2 Inhibitors

MK-8353 (SCH900353)

- Reported from a single phase I trial involving patients with advanced solid tumors.

- ORR: 12%.

- Responses were observed in patients with metastatic *BRAF* V600-mutant melanoma [103].

Ravoxertinib (GDC-0994)

- Results published from a single phase I trial conducted in patients with advanced solid tumors.

- ORR: 4%.

- Responses occurred in patients with metastatic *BRAF*-mutant colorectal cancer [104].

Ulixertinib (BVD-523, VRT752271)

- Evaluated in four clinical trials, primarily phase II, involving patients with advanced solid tumors, including uveal melanoma and acute myeloid leukemia (AML).

- ORR outcomes:

- 13.9% in a phase I trial targeting patients with solid tumors harboring *NRAS*, *BRAF* V600, and non-V600 *BRAF* mutations [105].

- 0% in a phase II trial involving patients with mutations in MAP kinase genes [106].

- Best outcomes:

- Disease stabilization in 15% of patients with high- and low-grade gliomas containing *BRAF* fusion or *BRAF* V600E mutations [106].

Summary

- p38 Inhibitor Ralimetinib showed minimal activity as monotherapy, with only disease stabilization observed.

- ERK1/2 Inhibitors MK-8353 and Ravoxertinib demonstrated limited efficacy, with low ORRs primarily in patients with specific genetic profiles (e.g., *BRAF* mutations).

- Ulixertinib yielded slightly higher activity in a phase I trial but lacked significant efficacy in phase II trials targeting genetically defined tumors, with ORR reaching 13.9% at best.

These findings emphasize the challenges of achieving meaningful clinical efficacy with ERK1/2 and p38 inhibitors as monotherapies and underscore the need for further investigation, potentially in combination regimens or stratified patient populations based on molecular profiles.

The ERK1/2 inhibitor Selumetinib (AZD6244) has been extensively studied in clinical trials, functioning as an indirect ERK1/2 inhibitor by suppressing its upstream activators, MEK1/2. Despite this, Selumetinib demonstrated limited efficacy across most trials.

Efficacy Overview

- Advanced Solid Tumors:

- ORR ranged from 0% to 7.1% in phase I and II trials NCT00888134 and [107–109].

- Ineffective Indications:

- Colorectal Cancer: ORR 0% [110,111].

- Pancreatic Cancer: ORR 0–5% [112,113].

- Sarcoma: ORR 5.9% [114].

- B-cell Lymphoma: ORR 0% [115].

- Limited Efficacy (ORR <30%):

- Metastatic Uveal Melanoma: ORR 14% [116].

- *BRAF* V600E Mutant Melanoma: ORR 20% in a subgroup with low phosphorylated AKT levels (5 patients) [117].

- Cholangiocarcinoma: ORR 12% and 15% in two trials [118,119].

- Serous Ovarian/Peritoneal Cancer: ORR 15% [120].

- High Efficacy in Pediatric Populations:

- Low-Grade Gliomas in Children: ORR 36–40%.

- Neurofibromatosis Type 1 and Inoperable Plexiform Neurofibromas: ORR exceeded 70%, leading to FDA approval in 2020 for children aged two years and older [121].

Safety Profile

For ERK1/2 and p38 inhibitors, safety was assessed using treatment-related metrics such as the incidence of all adverse events, severe adverse events (grade 3–4), and therapy-related mortality (**Supplementary Table 1**).

- Adverse Events (AEs):

- Most AEs were mild to moderate (grade 1–2).

- Severe AEs (grade 3–4) were observed in less than 30% of patients across trials, with some exceptions:

- Unresectable Melanoma: Grade 3–4 toxicity in 56.7% of patients [122].

- B-cell Lymphoma: Grade 3–4 toxicity in 64% of patients [115].

- Therapy-Related Mortality:

- Most trials reported no therapy-related deaths.

- Therapy-related mortality ranged from 1% to 5% in seven trials [107,111,122–124].

- An elevated mortality rate of 8% was observed in trials involving relapsed multiple myeloma [125].

Selumetinib's efficacy varies widely depending on cancer type, with notable success in pediatric low-grade gliomas and plexiform neurofibromas, contrasting with limited efficacy in most adult solid tumors. Its safety profile reflects mild to moderate adverse events in the majority of patients, though higher toxicity rates were noted in certain malignancies like unresectable melanoma and B-cell lymphoma. These findings highlight the need for selective use of Selumetinib in cancers with clear efficacy indications while managing safety risks in vulnerable populations.

5.2 ERK1/2 and p38 Inhibitors in Combination Therapy

To evaluate the efficacy of inhibitors in combination therapy, guidelines from the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) were analyzed. These frameworks define clinically significant therapeutic effects based on improvements in overall survival (OS) and progression-free survival (PFS), particularly in comparative clinical trials.

ASCO Guidelines

ASCO recommends the following metrics for new therapeutic regimens in specific cancer types:

- Relative Increase in Median OS: At least 20%.
- Absolute Extension of OS: 2.5 to 6 months, depending on the cancer type (e.g., pancreatic, lung, colorectal, and breast cancers) [126].

For other tumor types, a similar approach has been proposed, including a relative improvement of 25% and/or an absolute increase of 2.5 months in PFS or OS compared to standard treatments [127].

ESMO Guidelines

The ESMO criteria assess clinical benefit based on:

- The therapeutic intent (curative or palliative).
- The primary efficacy parameter (PFS or OS) [128].

These criteria are particularly relevant for trials demonstrating statistically significant differences between treatment groups. However, they are not applicable to a study with no significant intergroup differences [128].

Combination Therapy with ERK1/2 and p38 Inhibitors

Among 45 clinical trials investigating ERK1/2 and p38 inhibitors in combination with chemotherapy agents:

- 13 trials included a control group (**Supplementary Table 2**).
  - p38 Inhibitor Trial:
    - Ralimetinib dimesylate (LY2228820) combined with gemcitabine and carboplatin in patients with ovarian cancer.
  - ERK1/2 Inhibitor Trials:
    - Selumetinib (AZD6244) was explored in combination therapies for cancers such as breast cancer, biliary tract cancer, pancreatic cancer, thyroid cancer, melanoma, and non-small cell lung cancer (NSCLC).

Implications for Clinical Significance

The ASCO and ESMO criteria are essential tools for assessing the clinical relevance of combination therapies. For trials involving ERK1/2 and p38 inhibitors:

- Studies should demonstrate a statistically significant improvement in OS or PFS compared to control arms.
- Achievement of thresholds such as a 20% increase in OS or an extension of 2.5 months or more in OS/PFS would define clinically meaningful outcomes.
- The inclusion of control groups in combination trials enhances the reliability of comparisons and helps determine whether these inhibitors provide added benefit over standard therapies.

Future analyses should incorporate these criteria to refine the evaluation of combination therapies involving ERK1/2 and p38 inhibitors, particularly in cancers with unmet therapeutic needs.

In recent clinical trials, combinations involving the p38 inhibitor Ralimetinib dimesylate (LY2228820) and the ERK1/2 inhibitor Selumetinib demonstrated varying degrees of efficacy in improving progression-free survival (PFS), overall survival (OS), and objective response rates (ORR), with outcomes dependent on specific patient subgroups, cancer types, and mutation profiles. Below is a summary and analysis of key findings:

p38 Inhibitor Ralimetinib (LY2228820)

- Phase I Trial (Ovarian Cancer):
  - Combination: Ralimetinib + Gemcitabine + Carboplatin
  - Outcome: Statistically significant increase in PFS of approximately 2.4 months [129].
  - Relevance: Approaches the clinical significance threshold proposed by Kumar *et al.* [127] but highlights the need for further trials to confirm its efficacy.

ERK1/2 Inhibitor Selumetinib (AZD6244)

Combination with Docetaxel (NSCLC, *KRAS*-mutant)

- Phase II Trial:
  - PFS: Increased by 3.2 months, meeting the Kumar *et al.* [127] criteria but falling short of ASCO's 4-month threshold for NSCLC [130].
  - OS: Increased by 4.2 months; however, the result was not statistically significant.
  - ORR: 37% partial response rate.
  - KRAS Subgroup Analysis:
    - Patients with *KRAS* G12C or G12V mutations (MG1) showed greater benefit:
      - PFS: +4.3 months.
      - OS: +5.2 months.
      - ORR: 46%.
    - Patients with other *KRAS* mutations showed more modest improvements:
      - PFS: +2.3 months.
      - OS: +1.5 months.
      - ORR: 26% [131].
- Phase III Trial:
  - Results: No significant differences in PFS or OS compared to control.
  - ORR: 20.1% vs. 13.7% in control ($p = 0.05$, approaching significance).
  - No significant differences observed across *KRAS* mutation subgroups [132].

Combination with Docetaxel (Non-*KRAS*-mutant NSCLC)

- Phase II Trial:
  - Results were less promising:
    - No significant improvements in PFS or OS.
    - Modest increase in ORR to 18%, consistent with other trials [133].

Combination with Pemetrexed + Platinum Agents (NSCLC, Non-*KRAS*-mutant or Unknown *KRAS* Status)

- Phase II Trial:
  - PFS: Increased by ~3 months.
  - ORR: Increased by 43%.
  - OS: Decreased by 5 months compared to the control group.
  - None of these results reached statistical significance [134].

Insights and Implications

1. Combination Therapies with Ralimetinib:
   - The modest improvement in PFS with Ralimetinib highlights its potential but also emphasizes the need for further evaluation in larger and later-phase trials.
2. Selumetinib and Docetaxel in *KRAS*-Mutant NSCLC:
   - While *KRAS* G12C/G12V mutations appear to predict a better response, the lack of statistically significant OS improvements in phase III trials underscores the challenges of translating early promising results into clinical practice.
   - Limited efficacy in non-mutant *KRAS* populations suggests that *KRAS* mutations may be critical for patient selection.
3. Selumetinib in Non-Mutant/Unknown *KRAS* NSCLC:
   - The lack of significant efficacy in PFS and OS, combined with an unexpected reduction in OS when combined with pemetrexed and platinum agents, raises concerns about the broader application of Selumetinib in unselected NSCLC populations.
4. General Challenges in Combination Therapy:
   - While some combinations achieve meaningful ORR and PFS improvements, statistical significance and durability of benefits remain inconsistent across trials and subgroups.

The efficacy of p38 and ERK1/2 inhibitors in combination regimens varies significantly, often dependent on specific genetic contexts such as *KRAS* mutations. Moving forward, refining patient selection criteria and leveraging predictive biomarkers will be critical for improving outcomes and achieving clinically significant benefits in combination therapies.

In clinical trials, the ERK1/2 inhibitor Selumetinib has demonstrated varying efficacy in combination therapies, while results for other ERK1/2 inhibitors have been more limited. Below is a detailed summary of findings:

Selumetinib in Combination Therapy

1. Selumetinib + Dacarbazine (*BRAF*-Mutant Melanoma)
   - PFS: Statistically significant increase of 2.6 months, meeting Kumar *et al.*'s criteria [127].
   - ORR: Non-significant increase of 14%.
   - OS: Non-significant increase of 3.9 months [122].
2. Selumetinib + Other Agents (No Significant Improvements)
   - In remaining trials, combinations of Selumetinib with other agents failed to demonstrate statistically or clinically significant improvements across PFS, ORR, or OS compared to controls (**Supplementary Table 2**).
3. Combinations Showing Potential (No Control Group)
   - For NSCLC:
     - Gemcitabine + Cisplatin: ORR 30% [135].
     - Carboplatin + Paclitaxel: ORR 37.5% [136].
     - Pemetrexed + Cisplatin: ORR 30.4% [136].
   - For Thyroid Cancer:
     - Iodine-124: ORR 62.5%, based on a small cohort of 8 patients [137].

Other ERK1/2 Inhibitors in Combination Therapy

1. MK-8353 (SCH900353) + Selumetinib
   - Patient Population: Advanced solid tumors.
   - ORR: 0%, demonstrating no efficacy [138].
2. Ravoxertinib (GDC-0994) + Cobimetinib
   - Patient Population: Advanced solid tumors.
   - ORR: 6.7%, indicating limited clinical benefit [139].
3. Ulixertinib (BVD-523, VRT752271) + Gemcitabine + Nab-Paclitaxel (Metastatic Pancreatic Adenocarcinoma)
   - ORR: 20%, suggesting potential for further investigation, though only 5 response-evaluable patients were included.
   - PFS: 12.2 months.
   - OS: 5.5 months [140].

Selumetinib has shown modest clinical benefits in specific contexts, such as *BRAF*-mutant melanoma and certain NSCLC and thyroid cancer combinations (without control groups). However, efficacy remains inconsistent, and statistically significant improvements in outcomes are often lacking. Other ERK1/2 inhibitors, including MK-8353, Ravoxertinib, and Ulixertinib, have shown limited efficacy, with only Ulixertinib demonstrating a promising ORR of 20% in a small cohort of pancreatic cancer patients. Most promising combinations involved Selumetinib with standard chemotherapy agents in NSCLC and targeted therapy in thyroid cancer, highlighting the potential of ERK1/2 inhibitors in selected settings, particularly when paired with agents addressing complementary pathways.

Selumetinib remains the most extensively studied ERK1/2 inhibitor in combination therapy, but its efficacy is context-dependent, with limited clinical benefit in many cancers. The potential of other ERK1/2 inhibitors like Ulixertinib in specific combinations warrants further investigation in larger, controlled trials. Identifying biomarkers and stratifying patients based on genetic profiles, such as *BRAF* or *KRAS* mutations, may improve the therapeutic impact of these inhibitors in combination regimens.

The continued development of ERK1/2 and p38 inhibitors, alongside RAF and MEK inhibitors, is critical for

advancing cancer treatment strategies, especially in combination therapies designed to overcome resistance mechanisms. Such combinations leverage the complementary effects of targeted agents with immunotherapy, chemotherapy, or inhibitors targeting alternative pathways.

Combination Therapies in Development

1. RAF and MEK Inhibitors with Anti-PD-1 Immunotherapy

- Phase I Trial (NCT03543969):

- Combination of Encorafenib (RAF inhibitor), Binimetinib (MEK inhibitor), and Nivolumab (anti-PD-1 antibody).

- Patient Population: Stage III-IV melanoma harboring *BRAF* mutations.

- Outcome: Demonstrated synergy, highlighting the potential of integrating immunotherapy with MAPK pathway inhibitors.

2. Dual Targeting of RAS/RAF/MEK/ERK and PI3K/AKT/mTOR Pathways

- Synergistic Effects:

- Combining RAF or MEK inhibitors with AKT or mTOR inhibitors has shown potential in overcoming resistance in melanoma models.

- Mechanism:

- In vemurafenib-resistant melanoma models, this combination enhanced apoptosis, as evidenced by increased levels of cleaved caspase proteins detected via Western blotting [141].

Rationale for Combination Therapies

1. Overcoming Resistance:

- Resistance to monotherapies targeting MAPK pathways (e.g., vemurafenib, dabrafenib) frequently arises through reactivation of downstream signaling or activation of alternative pathways, such as the PI3K/AKT/mTOR axis. Combining inhibitors of these pathways addresses compensatory signaling mechanisms.

2. Enhancing Immune Response:

- MAPK pathway inhibitors can modulate the tumor microenvironment to enhance immune cell infiltration, making tumors more responsive to checkpoint inhibitors like anti-PD-1 therapies.

3. Synergy with Apoptotic Pathways:

- Dual inhibition promotes apoptosis by simultaneously blocking survival signals (e.g., AKT/mTOR) and proliferative signals (e.g., ERK), leading to enhanced cancer cell death.

Future Directions

1. Optimization of Combination Regimens:

- Refining dosing schedules and sequencing of MAPK inhibitors with immunotherapy or PI3K/AKT/mTOR inhibitors to maximize efficacy while minimizing toxicity.

2. Biomarker-Driven Approaches:

- Identifying patient subsets (e.g., specific *BRAF*, *KRAS*, or *PIK3CA* mutations) that are most likely to benefit from these combination strategies.

3. Expanding to Other Cancers:

- Extending these combination approaches beyond melanoma to include cancers where MAPK and PI3K pathway dysregulation are prevalent, such as colorectal, lung, and thyroid cancers.

The integration of ERK1/2 and p38 inhibitors with established therapies targeting RAF, MEK, and PI3K/AKT/mTOR pathways, along with immune checkpoint inhibitors, holds promise for overcoming acquired resistance and improving clinical outcomes. Ongoing trials and preclinical studies will provide further insights into optimizing these combinations for diverse cancer types. Recent studies underscore the therapeutic potential of targeting noncanonical regulators of the RAF/MEK/ERK pathway to overcome acquired resistance to targeted therapies in cancer [142,143]. Below are two promising approaches involving sirtuin 7 (SIRT7) inhibition in hepatocellular carcinoma (HCC) and the FXa-PAR2 axis in androgen-independent prostate cancer:

1. SIRT7 Inhibition in Sorafenib-Resistant Hepatocellular Carcinoma

- Mechanism of Resistance:

- Increased levels of SIRT7 stabilize the stress-responsive DDX3X protein by deacetylation at Lys55.

- DDX3X facilitates the assembly of the NLRP3 inflammasome, activating IL-1β signaling.

- IL-1β drives ERK1/2 phosphorylation, contributing to resistance against RAF/MEK/ERK inhibitors like sorafenib.

- Therapeutic Strategy:

- Two SIRT7 inhibitors were developed to disrupt this resistance mechanism:

- Inhibition of SIRT7 deactivates DDX3X and the NLRP3 inflammasome, suppressing IL-1β release and ERK1/2 signaling.

- Combination of sorafenib with SIRT7 inhibitors demonstrated:

- Synergistic reduction in tumor growth and proliferation.

- Significant suppression of ERK1/2 phosphorylation, re-sensitizing resistant cells.

- Implications:

- These findings support the further development of combination therapies targeting SIRT7 and RAF/MEK/ERK pathway inhibitors to enhance treatment efficacy in sorafenib-resistant HCC [142].

2. FXa Inhibition in Androgen-Independent Prostate Cancer

- Mechanism of Resistance:

- Activation of Factor X (FX) within the tumor microenvironment initiates the protease-activated receptor 2 (PAR2) signaling cascade.

- FXa-PAR2 signaling induces ERK1/2 phosphorylation, promoting androgen-independent tumor growth and reducing the effectiveness of the androgen receptor antagonist enzalutamide.

- Therapeutic Strategy:

- The FXa inhibitor rivaroxaban combined with enzalutamide was evaluated:

- *In vitro* and *in vivo* results showed that dual therapy significantly reduced tumor growth and proliferation compared to enzalutamide alone.

- Mechanism: Inhibition of FXa-PAR2 signaling suppressed ERK1/2 activation, overcoming resistance to androgen deprivation therapy.

- Implications:

- This strategy highlights the potential for targeting molecules outside the core RAF/MEK/ERK pathway that contribute to its signaling, offering an avenue for combating androgen-independent tumor progression [143].

Future Directions

1. Combination Strategies:

- Targeting SIRT7-DDX3X-ERK1/2 and FXa-PAR2-ERK1/2 pathways highlights a broader approach to overcoming resistance by disrupting auxiliary mechanisms that amplify ERK1/2 signaling.

- These combinations (e.g., sorafenib + SIRT7 inhibitors and rivaroxaban + enzalutamide) exhibit synergistic efficacy and merit further investigation in clinical trials.

2. Expanding Targets Beyond Core Pathways:

- These findings encourage the exploration of additional noncanonical regulators of the RAF/MEK/ERK pathway, particularly those involved in the tumor microenvironment or stress signaling.

3. Personalized Medicine:

- Patient stratification based on biomarkers such as SIRT7 levels or FXa-PAR2 activation could enhance the precision of combination therapies, improving outcomes while minimizing off-target effects.

By integrating these insights into clinical practice, these innovative strategies could improve responses to therapy and address key challenges in drug resistance across diverse cancer types.

6. Approaches for Assessing Individual Variations in Tumors

A comprehensive strategy for identifying tumors amenable to targeted therapy with ERK1/2 and/or p38 kinase inhibitors involves assessing the expression levels of the corresponding genes, leveraging advanced omics technologies, and applying sophisticated analytical approaches to interpret the data. This approach aligns with the principles of personalized medicine, where variability in gene expression supports tailoring treatments to individual patients [144,145].

Functional Enrichment Analysis for Gene Expression

Omics technologies enable simultaneous analysis of thousands of genes and biomarkers, facilitating the identification of relevant signaling or biochemical pathways. Two widely used approaches for functional enrichment analysis are [146–148] (Table 1):

1. Over-Representation Analysis (ORA)

- Compares differentially expressed genes (DEGs) exceeding a predefined threshold to curated gene sets representing pathways.

- Statistical tests determine whether the number of DEGs in a gene set is significantly higher than expected by chance [148].

2. Functional Class Scoring (FCS)

- Assigns an expression score to each DEG and evaluates whether the score deviates significantly (positively or negatively) from random expectations.

- Example: Gene Set Enrichment Analysis (GSEA), which uses permutation testing to assess statistical significance [146,149].

Challenges in Pathway Analysis

Functional enrichment tools often overlook the complexity of molecular networks, where alterations in gene expression may affect multiple pathways simultaneously. Key challenges include:

- Diverse Roles of Pathway Components: Some genes activate pathways, while others inhibit them.

- Feedback Regulation: Positive and negative feedback systems can significantly modulate pathway outcomes.

- Incomplete Analysis: Tools focused solely on gene expression may fail to account for the interplay of activators, repressors, and neutral components [150,151].

Quantitative Pathway Activation Analysis

To address these limitations, a quantitative approach has been developed to evaluate the activation level of entire molecular pathways, integrating their structure and functional components (Table 1).

Pathway Activation Level (PAL)

- Definition: A metric that quantitatively characterizes the activation or repression of a molecular pathway based on changes in gene expression [151,152].

- Calculation:

- PAL is computed as a weighted sum of the logarithms of case-to-normal expression ratios for all genes within the pathway.

- Weights are assigned based on each gene product's role in the pathway:

- Activator: Positive weight (+1).

- Repressor: Negative weight (–1).

- Neutral or ambiguous: Zero weight [153].

Table 1. Advantages and disadvantages of methods for assessing individual variations in tumors.

	Advantages	Disadvantages
Over-Representation Analysis (ORA)		
Simplicity	Easy to implement and interpret	May miss subtle or complex relationships between genes and pathways
Speed	Computationally efficient, making it suitable for large datasets	Relies on predefined gene sets, which may not capture novel or less-studied pathways
Interpretability	Results are straightforward, showing whether specific pathways or gene sets are over-represented	False positives can occur due to multiple testing. Ignores the magnitude of gene expression changes, focusing only on whether genes are present
Functional Class Scoring (FCS)		
Sensitivity	More sensitive to subtle changes in gene expression across a pathway	More computationally intensive than ORA
Integration of data	Takes into account the continuous nature of gene expression data, not just binary presence/absence	Interpretation can be more complex and influenced by the choice of scoring method and parameter settings
Flexibility	Better at dealing with noise and variability in gene expression data	More prone to false negatives if the signal is weak across the dataset
Pathway Activation Level (PAL)		
Pathway-level insights	Assesses the activation level of entire pathways, capturing the impact of gene expression changes across multiple pathways simultaneously	May require extensive knowledge of pathway interactions
Network-based analysis	Considers genes as part of complex molecular networks, reflecting the true biological context	More computationally intensive
Accuracy	Can detect and quantify the dysregulation of multiple pathways at once	May still miss novel interactions if the underlying network model is incomplete or inaccurate

Advantages of PAL:

1. Integrative Analysis: Accounts for the collective contribution of all pathway components, providing a holistic view.

2. Feedback Considerations: Incorporates pathway-specific regulatory dynamics, such as feedback loops.

3. Quantitative and Qualitative Insights: Combines numerical evaluation with biological interpretation, enhancing understanding of pathway regulation.

Applications of PAL in Cancer Research

1. Tumor Profiling for Targeted Therapy:

- Identifying tumors with high ERK1/2 or p38 pathway activation to determine suitability for kinase inhibitors.

- Assessing secondary pathway alterations (e.g., PI3K/AKT/mTOR) that could influence resistance or synergy.

2. Biomarker Discovery:

- Using PAL to correlate pathway activity with clinical outcomes, identifying predictive biomarkers for therapy response.

3. Therapeutic Optimization:

- Informing combination therapies by identifying co-dysregulated pathways that contribute to resistance or enhanced efficacy.

The development of advanced tools like PAL represents a significant step forward in understanding and targeting complex molecular networks in cancer. By integrating gene expression data with pathway architecture, researchers can derive a nuanced understanding of pathway activation, enabling personalized therapeutic strategies with inhibitors like ERK1/2 and p38 kinase inhibitors.

The Pathway Activation Level (PAL) method offers a comprehensive framework for evaluating the dysregulation of molecular networks in cancer research, providing insights into tumor biology and therapeutic responses. Below is an overview of the traditional PAL approach and its recently proposed gene-centric adaptation:

Traditional PAL Methodology

1. Node Annotation:

- Each component (node) in a molecular pathway is annotated as an activator or repressor based on its functional role and molecular interactions within the pathway architecture [151].

2. Algorithm for PAL Calculation:

- PAL is computed as a weighted sum of the logarithms of case-to-normal expression ratios for all genes in the pathway.

- Weights reflect the role of the gene product:

- Positive for activators.

- Negative for repressors.

- Neutral for components with ambiguous functions.

3. Key Features:

- Accounts for molecular interactions and feedback regulation.

- Mitigates distortions from varying data platforms and batch effects [154].

4. Applications:

- Differentiation of normal and tumor tissues [155].

- Prediction of therapeutic responses, with demonstrated utility in colorectal, kidney, and gastric cancers [156–159].

Gene-Centric PAL Approach

1. Concept:

- Constructs gene-centric pathways as networks centered around a specific gene of interest (e.g., ERK1, ERK2, p38α, p38β, p38γ, p38δ).

- Derived from the human interactome model, these pathways include maximum molecular interactions extending outward from the central node to other connected nodes.

2. Advantages:

- Focuses on the functional impact of central genes within a network, providing a highly specific assessment of pathway activity.

- Integrates large-scale interactome data to enhance pathway completeness and biological relevance.

3. Significance:

- Demonstrated prognostic and diagnostic utility, particularly in cancer research.

- Provides robust screening and predictive biomarkers for therapeutic responses [160,161].

Comparative Utility of Traditional and Gene-Centric PALs

- Traditional PAL:

- Offers a broad overview of pathway activity, particularly useful for identifying global dysregulation across multiple pathways.

- Gene-Centric PAL:

- Targets specific genes and their associated networks, enabling a more focused analysis with potential for higher predictive precision.

Applications in ERK1/2 and p38 Pathways

1. Assessing Tumor-Specific Activity:

- Gene-centric PALs for ERK1/2 and p38 pathways can differentiate between tumor and normal tissues by quantifying pathway activation or repression.

2. Predicting Therapy Response:

- PAL values can stratify patients based on pathway activity, guiding the selection of targeted therapies like ERK1/2 or p38 inhibitors.

3. Prognostic and Diagnostic Biomarkers:

- Gene-centric PALs serve as robust biomarkers for tumor aggressiveness and therapeutic sensitivity, aiding in personalized treatment planning.

The PAL method, particularly in its gene-centric form, is a powerful tool for integrating gene expression data with the functional roles of gene products in molecular pathways. Its application to ERK1/2 and p38 pathways provides a nuanced understanding of pathway dysregulation, offering significant prognostic, diagnostic, and therapeutic potential. As these tools are further refined, their adoption could greatly enhance precision oncology strategies.

7. Conclusions

This review underscores the critical molecular mechanisms governing malignant tumor proliferation, focusing on the biological roles of ERK1, ERK2, and p38 proteins, as well as their interactions with oncogenic signaling pathways. The practical implications of this research are significant, as it highlights the potential utility of evaluating:

- The prognostic and predictive value of ERK1, ERK2, and p38 expression levels.

- The quantitative ratios of these proteins.

- The activity of gene-centric signaling pathways associated with their regulation in carcinogenesis.

If validated in large-scale experimental and clinical studies, these insights could:

- Improve clinical outcome predictions, enabling more precise stratification of patients.

- Enhance anticancer therapy efficacy by guiding the development of tailored therapeutic strategies targeting these pathways.

By integrating molecular insights with clinical applications, this research paves the way for advancements in personalized oncology, optimizing treatment outcomes for patients with malignant tumors.

Author Contributions

AE, AM, AB and EP: Conceptualization, Analysis of literature, Writing–original draft and editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/FBL31317.

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