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MEK5-ERK5 Signaling in Cancer: Implications for Targeted Therapy

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Abstract

Mitogen-activated protein kinases (MAPKs) regulate diverse cellular processes including proliferation, cell survival, differentiation, and apoptosis. While conventional MAPK constituents have well-defined roles in oncogenesis, the MAPK kinase 5-extracellular signal-regulated kinase 5 (MEK5-ERK5) pathway has only recently emerged in cancer research. In this review, we consider the MEK5 signaling cascade, focusing specifically on its involvement in drug resistance and regulation of aggressive cancer phenotypes. Moreover, we explore the role of MEK5 in tumorigenesis and metastatic progression, discussing the discrepancies in preclinical studies and assessing its viability as a therapeutic target for anti-cancer agents.

Keywords

mitogen-activated protein kinase; MEK5-ERK5; cellular signaling; kinase inhibitors; targeted therapies

1. Introduction

The mitogen-activated protein kinase kinase 5-extracellular signal-regulated kinase 5 (MEK5-ERK5) pathway contains many features that are structurally and functionally distinct from other MAPKs, all of which increase its viability as a novel target for future

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therapeutics [1–3]. In the MAPK signaling network, MEK5 most resembles MEK1/2 by sequence alignment but remains the only known direct MEK activator of ERK5 [4]. MEK5 protein kinase is encoded by MAP2K5. Alternative splicing results in two isoforms of MEK5 (50 kDa α and 40 kDa β) differing in the N-terminus, which accounts for their relative binding affinities for ERK5 [5]. MEK5α contains a distinct docking site in its N-terminal extension, a phox and Bem1p (PB1) domain, crucial to ERK5 activation and transcriptional induction via myocyte enhancer factor 2C (MEF2C). Accordingly, MEK5α is a stronger activator of ERK5 than MEK5β, which lacks this consensus motif [6]. Moreover, the PB1 domain, present in all three components of this signaling cascade (Figure 1), acts as a scaffold to facilitate and maintain specificity of MEKK2–MEK5–ERK5 interaction and signaling [7].

Due to its extended C-terminus containing a nuclear localization signal (NLS), two proline-rich regions, and a transcriptional activation domain (TAD), ERK5, or big MAP kinase 1 (BMK1) encoded by the MAPK7 gene, is more than twice the molecular weight of other MAPKs (110 kDa). This structural distinction enables active ERK5 to undergo autophosphorylation of its C-terminal TAD, an ability unique to ERK5, thereby exerting direct control over gene transcription [8]. In the unphosphorylated state, ERK5 presents an inactive conformation, where its N- and C-terminal domains are associated together while in the cytosol. Activation by MEK5 induces an open conformation of ERK5, exposing the NLS, to relieve the autoinhibitory effects and facilitate ERK5 translocation to the nucleus [9–11]. ERK5 activity is also regulated by splice variants (a, b, and c) [12]. While ERK5a is the most highly expressed isoform, ERK5b and c, both deficient in protein kinase activity, can inhibit MEK5-mediated ERK5a stimulation.

Known substrates of ERK5 include transcription factors Sap-1a, c-FOS, c-MYC and MEF2 (A, C, and D) and kinases, such as RSK and serum/glucocorticoid-regulated kinase (SGK) (Figure 2) [13–17]. Similar to other proline (Pro)-directed MAPKs, ERK5 substrate recognition and subsequent phosphorylation occurs on amino acids Ser or Thr adjacent to a Pro residue (-X-Ser/Thr-Pro-X-sequence). Additionally, ERK5 protein kinase activity can be non-Pro-directed, as in the case of ERK5 autophosphorylation and ERK5-mediated MEK5 phosphorylation on Ser/Thr sites not directly preceding Pro residues [18]. These findings further distinguish ERK5 from other conventional MAPK family members.

2. Upstream activators of MEK5-ERK5 signaling

MEK5-ERK5 signal transduction can be activated by environmental stress, growth factors, and cytokines [13]. In response to these extracellular stimuli, MEKK2 or MEKK3 binds to the N-terminal domain of MEK5 and phosphorylates Ser311 and Thr315; however, the mechanisms of MEKK2/3 activation by external stimuli have not been fully elucidated [19]. MEKK2 has a higher binding affinity for MEK5 relative to MEKK3, but both MEKKs can also activate other conventional MAPK pathways, including JNK and p38 MAPK, via phosphorylation of their respective upstream MAP2Ks [20, 21]. Overexpression of MEKK2 has been detected in prostate and colorectal cancers, while elevated MEKK3 expression has been identified in breast, cervical, lung, kidney, and esophageal cancers [22–25].

MEKK2 is necessary for epidermal growth factor receptor (EGFR)- and human epidermal growth factor receptor 2 (HER2)-dependent activation of ERK5. Knockdown of MEKK2 inhibited tumor growth of triple-negative MDA-MB-231 and HER2-positive BT474 breast cancer xenografts and diminished metastasis of the TNBC cells [26]. MEKK2 has also been shown to regulate breast cancer cell migration by inducing focal adhesion turnover, specifically ubiquitylation and consequent removal of paxillin from focal adhesion complexes [27, 28]. To date, there are no selective MEKK2 inhibitors, though six compounds with potent *in vitro* MEKK2 inhibitory activity have recently been reported. Among this list of kinase inhibitors, Ponatinib (AP24534, Iclusig) is an FDA-approved drug indicated for BCR-ABL-targeting in treatment of chronic myeloid leukemia, suggesting its potential both as a preclinical research tool to elucidate the role of MEKK2 in cancer and as a drug repurposed for MEKK2-dependent cancers in the clinical setting [29].

The role of MEKK3 as a regulator of NF-κB signaling is well-documented [30, 31]. Overexpression of MEKK3 in glioma and ovarian cancer cells enhanced NF-κB activation and increased expression of cell survival factors to confer resistance to cytotoxic effects of chemotherapeutic agents [24, 32]. Conversely, silencing of MEKK3 by RNAi sensitized breast cancer cells to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) cytotoxicity through suppression of nuclear factor κB (NF-κB) transcriptional activity [33]. Furthermore, MEKK3 depletion induced cell death in renal cancer cells and reduced tumor growth of breast cancer cells, but did not significantly affect the frequency of metastasis [23, 26]. Despite their involvement in processes essential to tumorigenesis and malignancy, MEKK2/3 are understudied kinases. Instead, efforts have focused on parallel MEKKs and downstream effectors of MEKK2/3.

3. Pharmacological inhibitors of MEK5 cascade

Interest in the MEK5 pathway has emerged in cancer research partly due to its overlap with the MEK1/2 pathway along with the discovery that first-generation MEK1/2 inhibitors PD98059, U0126, and PD184352 also exhibit activity toward MEK5, providing impetus for the development of MEK5 selective inhibitors to parse the role of these pathways in cancer progression [10, 34]. The indolinone-6-carboxamides BIX02188 and BIX02189 (Boehringer Ingelheim Pharmaceuticals) were the first selective small-molecule ATP-site inhibitors of MEK5 signaling to be described, inhibiting MEK5 catalytic function with IC_{50} 4.3 and 1.5 nM, respectively [35]. BIX02189 also displayed more potent suppression of ERK5 kinase activity with IC_{50} 59 nM compared to that of BIX02188 (810 nM). Both compounds also inhibited transcriptional activity of MEF2, a downstream substrate of the MEK5 signaling cascade, in a dose-dependent manner. These MEK5 inhibitors blocked ERK5 phosphorylation without affecting activation of ERK1/2, p38 MAPK, or JNK [35].

Synthesis of XMD8-92 stemmed incidentally from a screen of analogs of BI-2536, a highly selective, ATP-competitive polo kinase inhibitor [36]. XMD8-92 selectivity for ERK5 was validated through profiling first against a diverse panel of 402 kinases and then against all detectable kinases in HeLa cell lysates, identifying ERK5 as most potently inhibited target with IC_{50} of 1.5 μM. MEK5 and ERK1/2 were not inhibited by XMD8-92, but the compound did significantly reduce ERK5-dependent MEF2C-driven gene expression.

Pharmacokinetics and tolerability of XMD8-92 was also evaluated in Sprague-Dawley rats. A single intravenous or oral dose of XMD8-92 was found to have a 2-hour half-life clearance of 26 mL/min/kg and high oral bioavailability with 69% dose absorption. After a single oral dose of 2 mg/kg, maximal plasma concentrations reached 500 nM within 30 minutes, with 34 nM remaining 8 hours post drug administration. To assess tolerability, plasma concentrations of XMD8-92 were maintained at high levels, 10 μM following IP dosing of 50 mg/kg, for 2 weeks. Animals did not show signs of morbidity or mortality [36].

TG02, an oral pyrimidine-based multi-kinase inhibitor, blocks CDKs 1, 2, 3, 5, and 9 with IC_{50} values below 10 nM in addition to janus kinase 2 (JAK2), p38δ, and ERK5 with IC_{50} values of 19, 56, and 43 nM, respectively [37–39]. The pharmacokinetic profile showed drug levels retained in tumors were above the IC_{50} for 8 and 24 hours after a single oral dose of 30 or 60 mg/kg, respectively [38]. TG02 treatment was well-tolerated in mice, even at maximum oral dosing of 40 mg/kg daily, with no body weight loss at endpoint. This novel anti-cancer agent has recently completed phase I of clinical trials for treatment of leukemia and multiple myeloma patients, the results of which may unveil the potential for MEK5 signaling inhibitors in cancer therapy.

4. Role of MEK5 pathway in drug resistance

Cytotoxic therapy

Drug resistance, both primary (intrinsic) and acquired, is a major obstacle in cancer therapeutics, indicative of more clinically aggressive tumor cells contributing to disease progression. The efficacy of cytotoxic agents used in chemotherapy, the standard-of-care for various cancer types, is mitigated by activation of signaling pathways, such as MEK5, that confer drug resistance [40]. Our lab and others have shown that MEK5 signaling promotes epithelial-to-mesenchymal transition (EMT), cell survival, and evasion of apoptosis – mechanisms linked to adaptive resistance [40–42].

Through expression profiling, we observed MEK5 upregulation in apoptotically resistant (APO−) MCF-7 breast cancer cell variants compared to apoptotically sensitive (APO+) cells. Transfection of dominant-negative (DN) ERK5 plasmid into APO− cells reduced cell viability in a dose-dependent manner versus vector control, and the cytotoxic effects of DN-ERK5 expression were augmented by treatment with apoptotic-inducing agents etoposide, tumor necrosis factor (TNF), or TNF-related apoptosis-inducing ligand (TRAIL). Furthermore, phorbol ester (PMA) stimulation failed to rescue cell viability of DN-ERK5-transfected cells treated with TRAIL [43]. In basal-like breast cancer subtypes, overexpression of MEK5 in conjunction with ERK5 was associated with poor relapse- and metastasis-free survival in patients who received chemotherapy compared to patients not treated with chemotherapy, which suggests that MEK5-ERK5 expression could serve as a predictive marker for patient benefit from systemic treatments in the ER-negative breast cancer setting [44]. Moreover, in MDA-MB-231 cells ERK5 inhibition by TG02 augmented anti-cancer effects of chemotherapeutic agents conventionally used in triple-negative breast cancer (TNBC) treatment, including taxotere, vinorelbine, and cisplatin [45]. These results support the role of MEK5 signaling in regulation of survival and apoptosis and implicate MEK5 pathway involvement in chemoresistance [43].

The pyrimidine analog 5-fluorouracil (5-FU), a widely used chemotherapeutic agent, is the common backbone of all standard polychemotherapy regimens for colorectal cancer [46]. While clinical efficacy of 5-FU exceeds that of other drugs, only 30% of colon cancer patients initially respond to therapy and the majority of which will develop resistance [47]. *In vitro* treatment of colon cancer cells HCT116 and SW620 with 5-FU reduced activation of both MEK5 and ERK5. Constitutive activation of MEK5 conferred a survival advantage to HCT116 cells exposed to 5-FU compared to empty vector cells, whereas downregulation of MEK5 signaling, either by transfection of dominant-negative ERK5 construct or treatment with a highly-selective ERK5 inhibitor XMD8-92, enhanced sensitivity of HCT116 cells to 5-FU-induced cytotoxicity through stimulation of p53-dependent transcriptional activation of p21 and Puma. The anti-apoptotic effects of 5-FU treatment in conjunction with ERK5 inhibition were recapitulated *in vivo* using an HCT116 xenograft model. Combination therapy using 5-FU and XMD8-92 significantly increased apoptosis and reduced tumor burden in comparison to monotherapy of each compound [48]. Consistent with this study, ERK5 inhibition via XMD8-92 treatment combined with doxorubicin, another chemotherapeutic agent, demonstrated synergistic induction of p53 and promoted significant tumor regression in both HeLa cervical cancer cells and A549 lung cancer cells [49]. Furthermore, small hairpin RNA (shRNA)-mediated knockdown of ERK5, as a mirror of ERK5 pharmacological inhibition, sensitized HMESO malignant mesothelioma cells to doxorubicin *in vitro* and synergized with doxorubicin in enhancing anti-tumor activity compared to vector control [40]. These findings provide rationale for the application of MEK5 pathway inhibitors coupled with 5-FU- or doxorubicin-based chemotherapy to enhance therapeutic efficacy and potentially delay the onset of drug resistance.

Targeted therapy

Pursuit of mechanism-based, individualized therapeutics has led to the development of small-molecule inhibitors and monoclonal antibodies targeting key signaling molecules or networks that drive cancer progression. Targeted therapies, though diverse in their mechanisms of action, have not overcome the hurdle of drug resistance. MEK5 signal transduction has been implicated as a critical factor in mediating sensitivity to several targeted therapies.

Endocrine resistance, either *de novo* or acquired, is evident in up to 50% of patients on an antiestrogen regimen, the mainstay in treatment of estrogen receptor alpha (ER-α)-positive breast cancer. ER-α signaling is an integral component of breast cancer biology as well as an important molecular mechanism perverted in endocrine therapy resistance [50]. Our lab has demonstrated that overexpression of MEK5 in the antiestrogen-sensitive, ER-α-positive (ER+) MCF-7 cell line downregulated ER-α expression and transcriptional activity in an ERK5-dependent manner and increased clonogenic survival following endocrine treatment [51]. These results delineate the role of MEK5-ERK5 signaling in progression to a more malignant estrogen-independent phenotype.

In breast tumors positive for human epidermal growth factor receptor 2 (HER2) expression, anti-HER2 therapy, such as trastuzumab, has demonstrated clinical efficacy in the adjuvant

setting, yet approximately 20% of patients experience relapse [52]. High ERK5 expression in patients with HER2-positive breast cancer was associated with worse disease-free survival [53]. HER2-enriched breast cancer cell lines SKBR3 and BT-474 have been shown to express constitutively active ERK5 [54]. Downregulation of ERK5 expression or activation potentiated anti-proliferative effects of trastuzumab in BT-474 cells, indicating that pharmacological inhibition of ERK5 may enhance anti-cancer action of trastuzumab [53]. Moreover, ERK5 inhibitor XMD8-92 synergized with heat shock protein (Hsp90) inhibition, proposed as a therapeutic target in TNBC, to suppress breast tumor formation *in vivo* [55]. In another cancer model, expression of dominant-negative ERK5 increased sensitivity of myeloma cells to apoptosis induced by the proteasome inhibitor PS341. Furthermore, overexpression of ERK5 in these cells abrogated the effects of PS341 on cell death [56]. Taken together, these studies implicate the MEK5-ERK5 pathway as a fundamental component of drug resistance in cancer therapy. Defining the mechanisms by which MEK5 promotes a therapeutically resistant phenotype may provide insight for the next generation of potent anti-cancer agents.

5. Role of MEK5 pathway in tumorigenesis

The MEK5-ERK5 cascade has been emerging as an important mediator of cell proliferation through induction of cell cycle regulators, including cyclin D1, c-MYC, n-MYC, SGK, RSK2, and NF-κB [15, 57–62]. Through phosphorylation of MEF2 transcription factors, MEK5 has been shown to regulate the expression of c-JUN, a proto-oncogene vital to cell growth [14, 63]; moreover, the ERK5-MEF2 axis has been reported in activation of survival signaling [64]. It has also been demonstrated that ERK5 can phosphorylate S403 and T409 of tumor suppressor promyelocytic leukemia protein (PML) and inhibit its activity, thereby downregulating the induction of p21 expression and enabling cells to overcome the G1-S phase [36, 65]. Constitutive activation of MEK5 in prostate and colon cancer cell lines accelerated cell cycle progression and increased proliferation [48, 66, 67]. Similarly, ERK5 knockdown studies using RNA interference (RNAi) or pharmacological inhibition by XMD8-92 treatment delayed cell cycle progression and decreased proliferation in various cancer types (Table 1). There are, however, conflicting reports showing that in cell lines harboring K-Ras or B-Raf mutations neither MEK5 inhibition, via BIX02189 or dominant-negative (DN) construct, nor siRNA-mediated downregulation of ERK5 affected cell growth, suggesting that in this cell context ERK5 is a dispensable proliferative signal [48, 68]. Interestingly, these results were also shown in ERK5-amplified SNU449 and KYSE30 cells [68], directly contradicting previous research demonstrating that knockdown of ERK5 resulted in cell growth inhibition in the ERK5-dysregulated hepatocellular and esophageal cancer cells, respectively [69, 70]. Recent findings have presented delineations between kinase activity and transcriptional activity of ERK5 that may account for discrepancies in determining ERK5 regulation of cellular proliferative responses [71]. Through a noncanonical mechanism involving Hsp90 dissociation, cell division cycle 37 (Cdc37) overexpression induced nuclear translocation of catalytically inactive but transcriptionally active ERK5 and collaborated with overexpressed ERK5 to promote cell proliferation [72]. Another study showed that XMD8-92 exhibited off-target kinase activity on bromodomain-containing protein 4 (BRD4), and using ERK5-selective derivatives, suggested that

inhibition of ERK5 kinase activity was not responsible for XMD8-92-mediated anti-proliferative effects [73]. Further research is needed to elaborate on the nuclear function of ERK5 independent of its catalytic status.

Using a conditional ERK5 knockout mouse model, Hayashi *et al.* demonstrated that tumor cells inoculated subcutaneously into the right flank region of the animals exhibited impaired vasculature development and reduced tumor growth, suggesting the involvement of ERK5 in the regulation of tumor-associated angiogenesis as well as tumor formation [58]. Studies since then have supported the involvement of the MEK5/ERK5 pathway in cancer progression (Table 2). We showed that hyperactivation of MEK5 in ER+ breast cancer cells enhanced estrogen-independent tumorigenesis [51], while others observed that ERK5 overexpression supported prostate tumor growth [74]. The role of ERK5 in tumor formation was further established as its silencing by shRNA impaired growth of malignant mesothelioma, T cell leukemia, and hepatocellular carcinoma xenografts through regulation of pro-inflammatory cytokines or NF-κB signaling [40, 60, 63]. Moreover, XMD8-92 treatment decreased tumor volume of various cancer types [36, 48, 63]. TG02, a multi-kinase inhibitor that targets ERK5, has also been shown to be an efficacious anti-tumor agent in the multiple myeloma and breast cancer settings [37, 45]. Based on studies demonstrating that shRNA-mediated knockdown of ERK5 did not alter growth dynamics of triple-negative breast cancer xenografts [26, 75], the anti-proliferative effects have been proposed as an artifact of TG02 activity against CDK targets. However, partial silencing of ERK5 may not be sufficient to exert anti-tumor effects in certain cell lines. For example, 70% ERK5 inhibition in SNU449 cells decreased proliferation while not affecting apoptosis, whereas 90% reduction of ERK5 expression in KYSE30 cells resulted in suppression of cell growth and significant induction of cell death [70]. As the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein-9 (CRISPR/Cas9) knockout system has been widely adopted for precision genome editing, it would be a beneficial tool in delineating the involvement of MEK5/ERK5 in tumorigenesis. Despite the controversy surrounding this research arena, MEK5 signaling remains a viable therapeutic target and elucidation of this pathway may provide insights to stratify the anti-cancer armamentarium, especially in regard to neoplasms such as hepatocellular carcinoma that require novel molecular therapies.

6. Role of MEK5 pathway in metastatic progression

Dysregulated MEK5 signaling is associated with metastatic risk in prostate, breast, colon, kidney, bone, and oral cancers as well as less favorable survival outcome [51, 67, 77–81]. Molecular inhibition of ERK5 *in vitro* suppressed cell motility and invasion of liver, breast, and prostate cancer cells [63, 82] and decreased metastasis of breast cancer xenografts *in vivo* [26, 75]. Conversely, cancer cells overexpressing MEK5 or ERK5 exhibited a migratory and invasive phenotype [66, 74], denoted by an increase in tumor metastases [67, 77, 83].

Metastasis, a complex process in which malignant cells originating from the primary tumor infiltrate and colonize distal organs, is organized into simplified steps: local invasion, intravasation of cells into the circulation, dissemination, extravasation of cells at distant sites, and colonization. Epithelial-to-mesenchymal transition (EMT) is an integral part of

metastatic progression whereby cells adopt motile and invasive capabilities through loss of epithelial markers, namely Cadherin 1/E-Cadherin (CDH1), and acquisition of mesenchymal markers, such as vimentin (VIM) and Cadherin 2/N-Cadherin (CDH2). MEK5 signaling has been implicated in the activation of EMT and transcription factors linked to EMT induction, including NF-κB and FOS-Like Antigen 1 (FRA-1) [16, 51, 67]. Furthermore, ERK5 signaling has been shown to regulate the expression of matrix metalloproteinase (MMP) family members (MMP-1, 2, 9, 12, and 16), known for their role in degradation of the extracellular matrix (ECM) to potentiate cancer cell dissemination [40, 66, 77, 84], and other proteins involved in migration and invasion, such as tissue inhibitor of metalloproteinases 2 (TIMP2) and bone morphogenic protein 5 (BMP5) [40, 77]. While many studies have presented a positive correlation between ERK5 expression and EMT induction, dissenting observations have been reported. Inhibition of ERK5 in A549 lung cancer cells by XMD8-92 treatment did not affect transforming growth factor-β1 (TGF-β1)-induced EMT, whereas BIX02189 abrogated the pro-metastatic effects of TGF-β1 surprisingly through suppression of TGF-β type I receptor (TβRI) activation, not MEK5/ERK5 signaling, although the level of MEK5/ERK5 activation was not determined [85]. In another investigation using metastatic A549 cells, knockdown of ERK5 resulted in reduced protein expression of CDH1 and ZO-1, upregulation of snail family zinc finger 1 (SNAI1), CDH2, and VIM, and enhanced cell migration [86]. Contrary to these findings, a recent study utilizing the same lung cancer cell line cited EMT suppressive, or MET inducing, effects of ERK5 depletion, including increased levels of CDH1 and reduction in cell migration, through regulation of SNAI2 with no change observed in SNAI1 levels [75]. These morphogenetic changes were recapitulated in a highly aggressive mesenchymal breast cancer model where suppression of ERK5 induced an epithelial phenotype and decreased intravascular invasion, leading to significantly fewer circulating tumor cells (CTCs) derived from primary orthotopic xenografts and reduction of metastatic lesions [75].

In addition to tumor cell intravasation, EMT has been linked to enrichment of the cancer stem cell (CSC)-like phenotype, further cementing its role in the metastatic cascade [87]. CSCs exhibit tumor-initiating potential, vital for metastatic colonization, attributed to their ability to self-renew and generate differentiated progeny that do not bear CSC cell-surface markers. From the multitude of studies establishing connections between MEK5 signaling and EMT, it follows that ERK5 would be involved in regulation of CSCs. Indeed, ERK5 activation was associated with enhanced CSC tumor sphere formation and tumor-initiating capacity [88]. Inhibition of ERK5 abrogated the effects of MEK5 activity on tumorigenicity of A549 spheres through hypoxia-inducible factor 1α (HIF1α)-mediated upregulation of apoptosis-associated genes BCL2 interacting protein 3 (BNIP3) and BNIP3 like (BNIP3L).

Involvement of the MEK5 pathway has also been described in disruption of actin dynamics leading to alterations in cell migration/invasion potential and metastatic dissemination. For instance, transfection of ERK5 expression construct in prostate cancer cells promoted formation of invadopodia, actin-rich protrusions of the plasma membrane associated with increased invasiveness of cancer cells [77]. Additionally, novel roles of ERK5 have been demonstrated in cytoskeletal remodeling pathways. PMA-stimulated ERK5 activity was implicated in regulation of cell morphology through phosphorylation of focal adhesion kinase (FAK) on S910 [89]. Moreover, integrin-mediated FAK signaling was linked to

ERK5 activation in prostate and breast cancer cells, resulting in enhanced cell motility [82]. The ER-α/ERK5/cofilin (CFL1) network is another regulatory pathway of actin organization. While it has previously been shown that MEK5 signaling represses ER-α expression in breast cancer cells thereby promoting a more malignant hormone-independent phenotype, the role of ER-α was recently discovered in nuclear recruitment of ERK5 and CFL1, restricting their colocalization to cytoplasmic regions of actin remodeling, to suppress metastatic capacity [51, 90]. Notably, in ER-negative cell lines introduction of ER-α or ERK5 inhibition using XMD8-92 impaired cell motility and invasiveness [90].

Cell division cycle 42 (Cdc42), a member of the Rho GTPase family was shown to exert breast cancer cell line-specific effects on metastatic potential in part through regulation of the ERK5 pathway. It was reported that knockdown of Cdc42 increased ERK5 phosphorylation and suppressed cell motility and invasion in moderately metastatic Hs-578T breast cancer cells, suggesting that ERK5 signaling negatively correlates with metastatic progression [91, 92]. However, Cdc42 depletion enhanced cell migration and invasion in highly aggressive MDA-MB-231 breast cancer cells [91]. If activation of ERK5 associated with Cdc42 silencing, then ERK5 would exert pro-metastatic effects in these highly invasive cells; yet ERK5 activity was shown to decrease the invasive potential of MDA-MB-231 cells [92]. These conflicting results highlight the nuanced and cellular context-dependence of ERK5 function in modulating the invasive phenotype and further supports continued investigation of MEK-ERK5 signaling in regulation of metastatic progression.

7. Future perspective

Conventional MAPK family members, such as MEK1/2, are currently undergoing clinical trials, evaluated by potential to reduce tumor burden and improve progression-free survival in advanced-stage cancers. Studies have also assessed combinations of MEK1/2 inhibitors and other targeted agents or cytotoxic chemotherapy aimed at mitigating resistance mechanisms and enhancing patient response [45, 93, 94]. In particular, trametinib, a MEK1/2 inhibitor, has exhibited anti-tumor activity in the treatment of BRAF-mutated melanoma and has gained FDA approval as both a stand-alone agent and in combination with BRAF inhibitor dabrafenib [95, 96].

Recent advancements in unravelling the role of MEK5-ERK5 signaling in oncogenesis have shed light on its potential as a target in novel cancer therapeutics. Deregulation of the MEK5 pathway has been implicated in metastatic prostate cancer, colon cancer, and invasive osteosarcoma, demonstrating its broad range of application across various cancer types [66, 67, 97]. Elevated levels of ERK5 expression and activity correlates with worse prognosis in patients with triple-negative breast cancer, an aggressive subtype for which there are currently no targeted therapies available [45, 51]. Preclinical studies have shown that inhibition of the MEK5 cascade decreased intravascular invasion leading to decreased circulating tumor cells and formation of metastatic lesions, implicating its role in tumor progression and metastasis. Moreover, MEK5 signaling is strongly linked to chemoresistance. Overall, our work as well as others highlight the importance of this understudied pathway in cancer biology.

Due to redundancies in the MAPK signal transduction cascade and high degree of overlap in downstream targets of the MEK1/2 and MEK5 pathways, further investigation is warranted in determining potential synergy of combined MEK1/2 and MEK5 inhibition in targeting aggressive cancer types to delay the onset of drug resistance and maximize patient response to therapy. Understanding the MEK5-ERK5 pathway will provide a pivotal stage to expand the current spectrum of MEK inhibitor therapies and lead to wider application of such treatments.

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- MAPKs regulate diverse cellular processes including proliferation, cell survival, differentiation, and apoptosis
- The MEK5-ERK5 cascade has emerged as an important mediator of tumorigenesis and metastatic progression
- These studies implicate the MEK5-ERK5 pathway as a fundamental component of drug resistance in cancer therapy

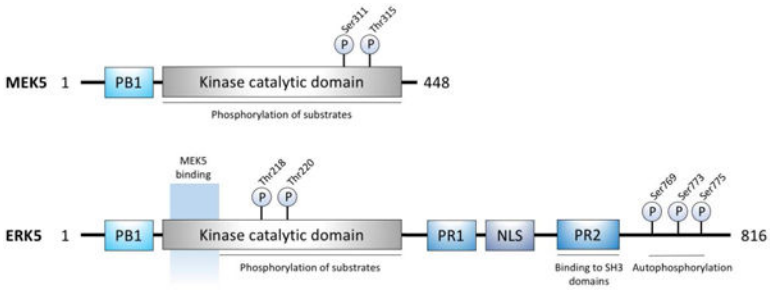

Figure 1. Structure of MEK5 signaling components

Linear representation of MEK5 and ERK5, PB1 - Phox and Bem1p, PR1 – Proline rich domain 1, NLS – Nuclear localization, PR1 – Proline rich domain 2

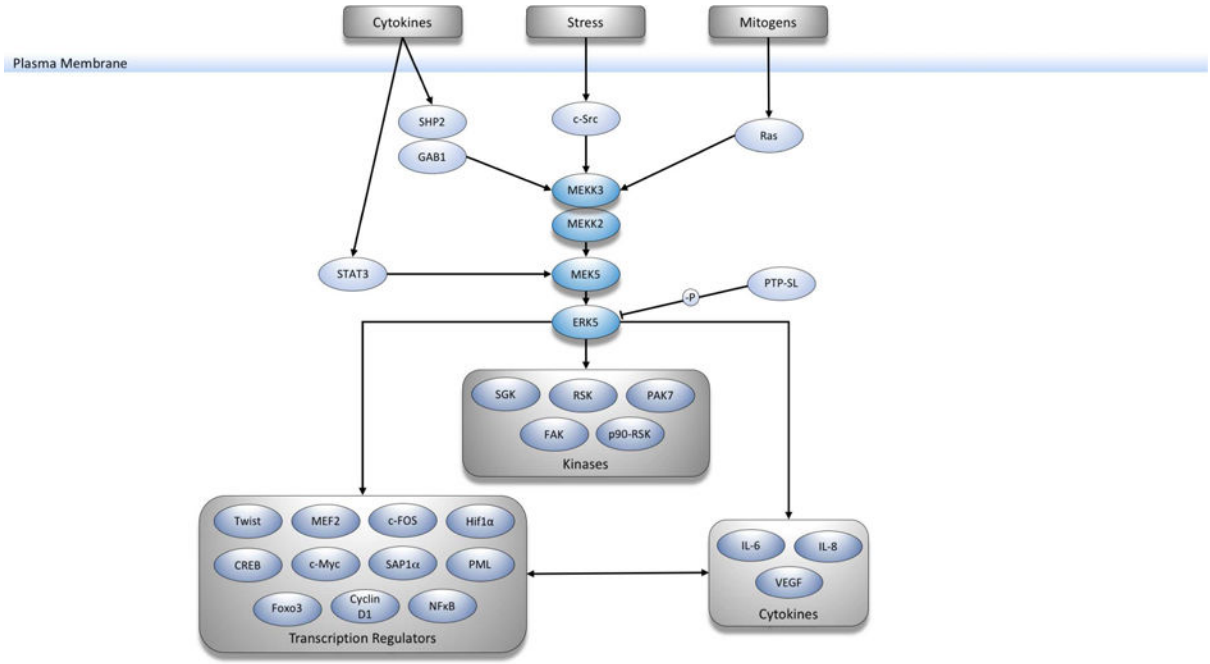

Figure 2. MEK5 activation and downstream substrates

MEK5/ERK5 pathway can be activated by stress, mitogens or cytokines, leading to the regulation of various downstream targets including kinases and transcription factors.

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Table 1

Effects of MEK5/ERK5 signaling on *in vitro* cancer cell proliferation.

Disease	Cell line	Targeted Approach	Effects (compared to control)	Mechanism	Ref.
T cell leukemia	Jurkat T	shRNA	did not affect cell cycle progression, sensitized cells to TNF-α	ERK5 activates NF-κB signaling and promotes nuclear localization and transcriptional activity of p65.	[60]
breast carcinoma	BT-549	shRNA	decreased cell proliferation	TG02 decreased expression of antiapoptotic proteins MCL1 and BCL2 and triggered cell death through caspase-dependent and -independent mechanisms.	[45]
	Hs-578T				
	HCC1187				
	MDA-MB-231				
	MDA-MB-231	TG02	delayed cell cycle progression, induced apoptosis		
	HCC1187				
prostate carcinoma	LNCaP	CA-MEK5	increased proliferative index	CA-MEK5 expression increased percentage of cell in S phase.	[66]
	PC3	overexpression	increased proliferative index	High levels of ERK5 is associated with accelerated cell cycle progression.	[74]
multiple myeloma	MM1S	TG02	suppressed cell cycle progression and induced apoptosis	TG02 activated apoptosis through intrinsic and extrinsic pathways.	[37]
cervical adenocarcinoma	HeLa	DN-ERK5 (Ala-Glu-Phe [AEF])	inhibited proliferation	ERK5 inhibited PML function and p21 expression to regulate cell proliferation.	[36]
		XMD8-92			
lung carcinoma	A549	XMD8-92			
	NCI-H1793	siRNA	did not affect cell proliferation or death	Dysregulated ERK5 signaling drives cell proliferation.	[70]
esophageal carcinoma	KYSE30	siRNA	decreased proliferation, increased cell death		
hepatocellular carcinoma	SNU449	siRNA	decreased proliferation		
hepatocellular carcinoma	HepG2	siRNA	decrease in proliferation	XMD8-92 treatment decreased expression of cyclin D1.	[63]
	Huh-7				
	HepG2	XMD8-92	decrease in proliferation, reduction of cells in S phase and increased percentage of cells in G0/G1, no indications of apoptosis		
	Huh-7				
	SNU449	siRNA	inhibited cell growth	RNAi knockdown of ERK5 decreased mitotic index, implicating ERK5 involvement in regulation of mitotic entry.	[69]
	SNU449	siRNA	inhibition of ERK5 expression or activity did not decrease proliferation	K-Ras/B-Raf-mutated or ERK5 amplified cancer cells are not dependent on MEK5 pathway for proliferation.	[68]
		BIX02189			

Disease	Cell line	Targeted Approach	Effects (compared to control)	Mechanism	Ref.
colon carcinoma	HCT116	siRNA	did not affect proliferation		
		BIX02189	inhibited proliferation at high doses (>10 μM)		
	HT29	BIX02189	inhibited proliferation at high doses (>10 μM), did not affect proliferation in 3D culture		
	HCT116	DN-MEK5	did not affect proliferation index (however, ERK5 overactivation by CA-MEK5 increased cell proliferation)	ERK5 inhibition was associated with increased p53 transcriptional activity, upregulating p21 and Puma.	[48]
	SW620		did not affect proliferation index		
	SW620	DN-MEK5	accelerated cell cycle progression in CA-MEK5 cells, which was abolished by XMD8-92 treatment	MEK5 signaling promotes cell cycle progression through degradation of IκB leading to NF-κB activation.	[67]
		CA-MEK5			

Note: ERK5 inhibition; MEK5 inhibition

Table 2

Effects of MEK5/ERK5 signaling on *in vivo* tumorigenesis.

Disease	Cell line	Targeted Approach	Effects (compared to control)	Ref.
lung carcinoma	LL/2	deletion of host gene	delayed tumor development, reduced tumor vasculature	[58]
melanoma	B16F10			
prostate carcinoma	PC3	overexpression	enhanced tumor formation	[74]
breast adenocarcinoma	MCF7	CA-MEK5	enhanced tumor growth independent of estrogen, shRNA-downregulation of ERK5 decreased MCF7-MEK5 tumor growth	[51]
	MDA-MB-231	TG02	delayed tumor growth	[45]
	MDA-MB-231	shRNA	did not significantly affect tumor growth	[26]
	MDA-MB-23◆	shRNA	did not significantly affect tumor growth	[75]
multiple myeloma	MM1S	TG02	inhibited tumor growth	[37]
	OPM2		inhibited tumor growth	
malignant mesothelioma	HMESO	shRNA	impaired tumor formation	[40]
	H2373			
T cell leukemia	EL-4	shRNA	impaired tumor formation	[60]
hepatocellular carcinoma	Huh-7	shRNA	suppressed tumor growth by 100-fold	[63]
		XMD8-92	suppressed tumor growth due to reduction in cell proliferation, no change in levels of apoptosis	
colon carcinoma	HCT116	XMD8-92	inhibited tumor growth by 46%	[48]
pancreatic adenocarcinoma	AsPC-1	XMD8-92	inhibited tumor growth and decreased tumor volume	[76]
cervical adenocarcinoma	HeLa	XMD8-92	suppressed tumor growth, blocked tumor cell proliferation	[36]
lung carcinoma	LL/2			

Note: ERK5 inhibition; MEK5 inhibition

◆(4175 TGL variant)