# The MEK5/ERK5 signalling pathway in cancer: a promising novel therapeutic target

André E.S. Simões, Cecília M.P. Rodrigues[1] and Pedro M. Borralho[1]

Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal

**Conventional mitogen-activated protein kinase (MAPK) family members are among the most sought-after oncogenic effectors for the development of novel human cancer treatment strategies. MEK5/ERK5 has been the less-studied MAPK subfamily, despite its increasingly demonstrated relevance in the growth, survival, and differentiation of normal cells. MEK5/ERK5 signalling has already been proposed to have pivotal roles in several cancer hallmarks, and to mediate the effects of a range of oncogenes. Accumulating evidence indicates the contribution of MEK5/ERK5 signalling to therapy resistance and the benefits of using MEK5/ERK5 inhibitory strategies in the treatment of human cancer. Here, we explore the major known contributions of MEK5/ERK5 signalling to the onset and progression of several types of cancer, and highlight the potential clinical relevance of targeting MEK5/ERK5 pathways.**

## Introduction

The MAPK family members regulate signal transduction cascades that are highly conserved among eukaryotes and are known to be involved in the control of several intracellular events, including proliferation, differentiation, migration, and apoptosis (reviewed in [1]). Thus far, four conventional and three atypical MAPK subfamilies have been identified. The four conventional MAPK subfamilies are: extracellular signal-regulated protein kinases 1/2 (ERK1/2); c-Jun N-terminal kinases 1–3 (JNK1, 2 and 3); p38 MAPKs (p38 α, β, γ and δ); and the most recently discovered ERK5. By contrast, ERK3/4, ERK7, and Nemo-like kinase (NLK) are considered atypical MAPKs partly because their N- and C-terminal domain extensions are not similar to, nor present in, conventional MAPKs. Despite the approximately 45% homology to conventional MAPKs, the biological role of atypical MAPKs remains elusive [1].

Activation of conventional MAPK cascades is triggered by several stimuli, including internal metabolic stress, as well as by external mitogens, hormones, or neurotransmitters, cell–matrix and cell–cell interactions (reviewed in [2]). The well-stratified canonical activation of MAPK cascades proceeds through a three-tiered hierarchical module comprising a mitogen-activated kinase kinase kinase (MAPKKK) that phosphorylates mitogen-activated kinase kinase (MAPKK), which ultimately phosphorylates MAPK (reviewed in [3]) (Fig. 1).

## ERK5 identification

ERK5, encoded by the *MAPK7* gene, was first identified as big mitogen kinase 1 (BMK1) and cloned in two independent studies, two decades ago. It was found to be expressed in several tissues, being particularly abundant in heart, brain, lung, skeletal muscle, placenta, and kidney [4,5]. Structurally, ERK5 protein contains a N-terminal kinase domain with approximately 66% sequence homology with ERK1/2, and a unique and large C terminus that contains a transactivation domain, a nuclear localisation sequence (NLS), a nuclear export sequence (NES), and two proline-rich regions [4,6,7]. ERK5 activation occurs via dual phosphorylation of its N-terminal domain, prompted by active MEK5. Once active, ERK5 phosphorylates multiple sites in its C-terminal domain, enhancing its own transcriptional activity [8] (Fig. 2). In the unphosphorylated form, ERK5 presents an autoinhibitory mechanism, where its N- and C-terminal domains are bound. The activation of ERK5 N-terminal kinase by MEK5 allows the phosphorylation of ERK5 substrates and the ERK5 C-terminal

*Corresponding authors:* Rodrigues, Cecília M.P. (cmprodrigues@ff.ulisboa.pt), Borralho, P.M. (borralhopm@gmail.com)
[1] Senior authors.



1359-6446/
http://dx.doi.org/10.1016/j.drudis.2016.06.010

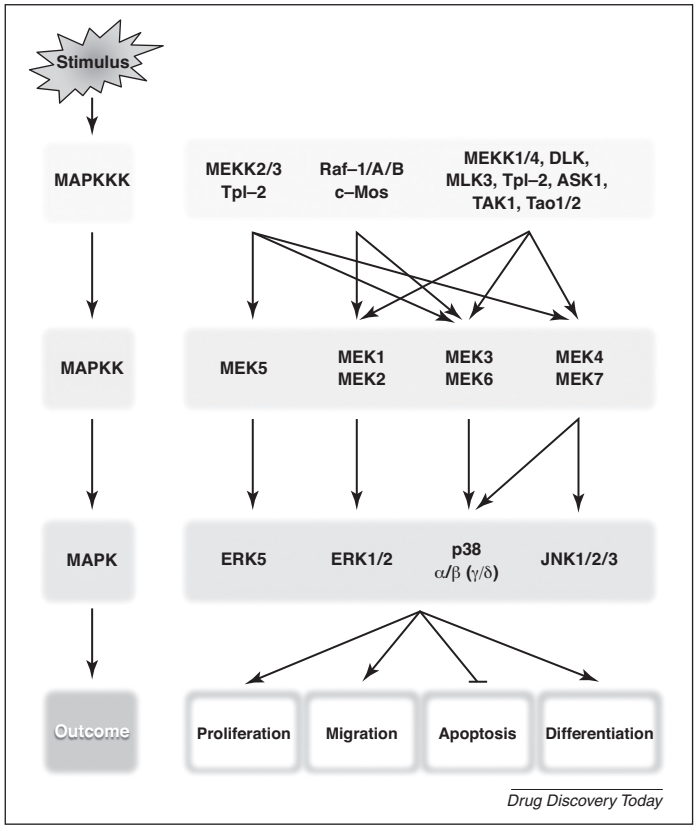


**FIGURE 1**

General schematic representation of conventional three-tiered mitogen-activated protein kinase (MAPK) signalling networks, which ultimately lead to activation of terminal MAPKs. MAPK kinase kinase (MAPKKK) activation following different stimuli, including mitogen, cytokine, or cellular stress, promotes the sequential activation of different MAPK kinases (MAPKKs). Subsequently, the specific activation of different MAPK signalling pathways will be effected by activated MAPKKs. Nevertheless, the known crosstalk within MAPK signalling networks, particularly at the MAPKKK level, allows the combined action of different terminal MAPK proteins. MAPK signalling pathways are typically involved in the regulation of several major cellular physiological roles, such as proliferation, migration, differentiation, and apoptosis.

domain. This latter phosphorylation abolishes any autoinhibitory effects, exposes the NLS domain, and determines the role of ERK5 signalling in the cell (reviewed in [6]).

Curiously, two studies unveiled a potential novel MEK5-independent ERK5 activation pathway that occurs during mitosis and relies on cyclin-dependent kinases (CDKs), being particularly relevant during the G2–M phase transition [9,10]. Beyond this discovery, a new cross-talk mechanism within MAPK family members has been proposed, in which active ERK1/2 phosphorylates ERK5 at the Thr732 residue in the C-terminal domain, without the typical activation of the N-terminal kinase domain, and is able to direct ERK5 to the nucleus [11] (Fig. 2).

## The physiological role of ERK5

ERK5 was initially identified as a MAPK that was generally activated by both oxidative and osmotic stresses [12]. Subsequent studies demonstrated that it is also activated by serum [13], a range of growth factors, including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) [14–17], and also by cytokines, such as leukaemia inhibitory factor (LIF) [18] and interleukin 6 (IL-6) [19].

Thus far, the MEK5/ERK5 signalling pathway has been implicated in the regulation of several cellular processes, including survival, antiapoptotic signalling, proliferation, angiogenesis, and differentiation (reviewed in [20]). However, to better address the physiological role of ERK5, several mouse knockout models with ablation of ERK5 signalling have been generated. The phenotypic abnormalities observed after *MEK5* ablation (*MEK5*$^{-/-}$) included impaired cardiac development, decreased proliferation, and increased apoptosis in heart, head, and dorsal regions that ultimately led to embryonic death [21]. Similarly, in *ERK5*$^{-/-}$mice, phenotypic abnormalities included general angiogenic defects in the embryo and placenta that led to underdeveloped vasculature, defective cardiac development, and increased embryonic endothelial cell apoptosis, ultimately resulting in embryonic death at embryonic day (E)9.5–E11 [16]. Furthermore, inducible *ERK5*-knockout mouse models were fatal by 2 weeks after birth, because of degeneration of the cardiovascular system with increased endothelial cell apoptosis [16]. In fact, the phenotypic outcome observed after specific ablation of ERK5 in endothelial cells was similar to that observed following general *ERK5* ablation [16]. However, when *ERK5* ablation was specific to cardiomyocytes, mice developed normally, but with increased cardiac vulnerability to hypertrophic stress, and were consequently demonstrated to be prone to undergo heart failure under intense workload conditions [22]. All these data suggest that ERK5 signalling is crucial for cardiovascular system development and vascular integrity maintenance, as well as for endothelial cell function.

## ERK5 in cancer development

MEK5/ERK5 signalling is one of the less-studied MAPK cascades. It has already been reported that this pathway has an important role in cell survival, proliferation, angiogenesis, motility, and differentiation, and in repressing the apoptosis of normal cells [20]. In cancer, there is increasing clinical evidence regarding the involvement of MEK5/ERK5 signalling in tumour development and progression.

Therefore, here, we discuss the increasing body of evidence implicating MEK5/ERK5 signalling pathways in the onset, progression, and therapy response in cancer types highly ranked in incidence, and explore the potential rationale for targeting MEK5/ERK5 as a therapeutic approach in cancer.

### *Breast cancer*

Breast cancer is the world's leading diagnosed noncutaneous cancer in women [23]. Based on gene expression profiles, breast cancer can be categorised in four major molecular subtypes: human epidermal growth factor receptor 2 positive (HER2+); hormone receptor positive (ER+ and/or PR+) (luminal A); both HER2+ (or HER2 low with Ki67 high) and hormone receptor positive (ER+ and/or PR+) (luminal B); and lastly, triple-negative breast cancers (TNBC) (HER2−, ER− and PR−) [24]. Luminal (A and B) tumours comprise 60% of breast cancer cases, whereas TNBC and HER2+ represent 15–20% and 10–15% of breast tumours, respectively. The clinical features across all subtypes are distinct because of their

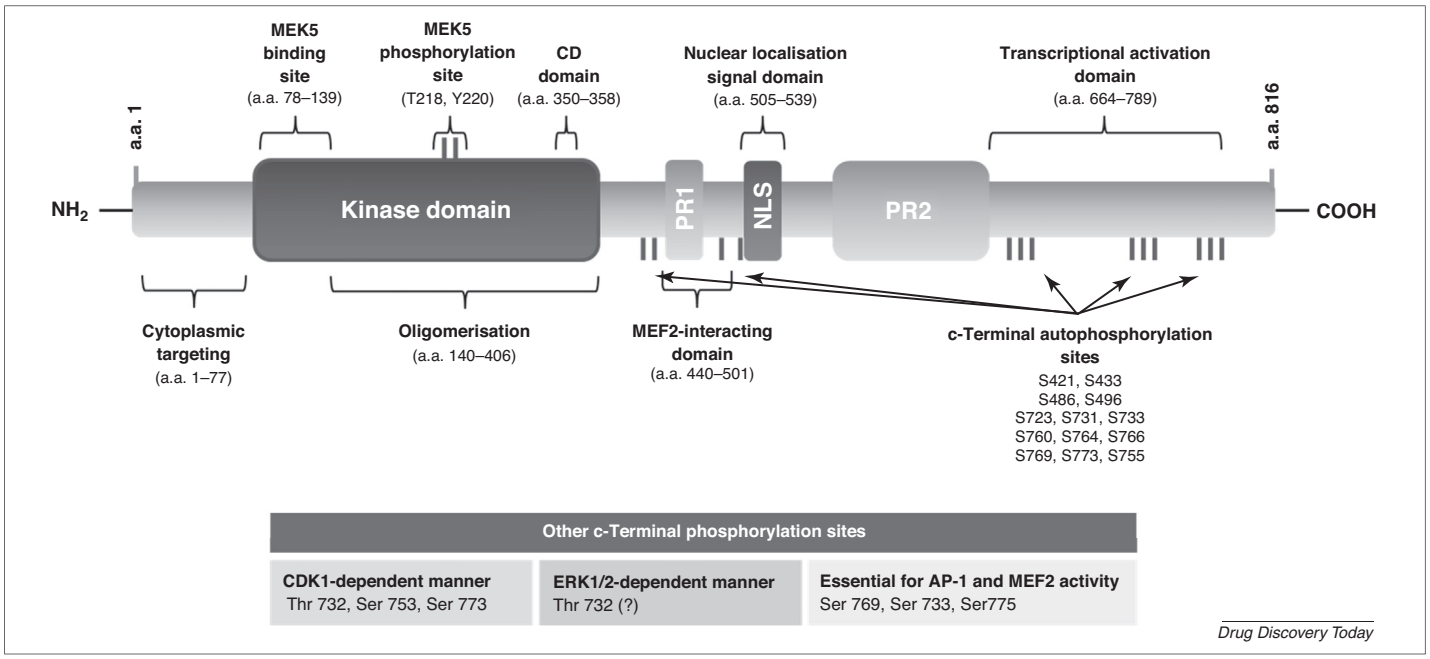


**FIGURE 2**

Schematic representation of ERK5 structure and functional domains. The ERK5 protein comprises 816 amino acid (a.a.) residues with a N-terminal domain and a large and unique C-terminal domain. The N-terminal domain contains the cytoplasmic targeting region, a kinase domain with two MEK5 phosphorylation sites, a common docking (CD) domain, and the oligomerisation region. The unique C-terminal domain contains two proline-rich (PR) domains, a MEF2-interacting region, the nuclear localisation signal (NLS) domain, and a transcriptional activation domain. The C-terminal domain also contains several different phosphorylation sites that are responsible for different physiological outcomes.
*Source*: Adapted from Refs. [7,11].

diverse genetic profiles [24,25]. Usually, luminal tumours respond better to endocrine therapy and chemotherapy compared to HER2+ and TNBC tumours, and are generally associated with a better prognosis [25].

Deregulation of the MEK5/ERK5 pathway in breast cancer was firstly associated with decreased overall survival in patients with HER2+ breast cancer [26]. Nonetheless, tumours from mice engineered to develop TNBC-like tumours also presented unusual high levels of ERK5 expression compared with normal breast tissue from the same mice [27]. In fact, MEK5 and ERK5 levels were found frequently increased either in TNBC or in HER2+ human breast tumours, and correlated with poorer relapse-free survival [27,28]. ERK5 overexpression was linked to poorer relapse-free survival in patients receiving chemotherapy, but not in patients not receiving chemotherapy. This led to the conclusion that patients with low ERK5 expression benefited from systemic treatments, whereas those with high ERK5 expression did not [29]. Regarding luminal tumours, MEK5/ERK5 signalling enhancement was associated with worse prognosis because of the contribution to endocrine treatment resistance [30] and progression to hormone-independent tumorigenesis [31].

In conclusion, MEK5/ERK5 signalling is a relevant potential therapeutic target in all breast cancer molecular subtypes. In addition, ERK5 inhibition through genetic approaches or chemical inhibition using TG02 or XMD8-92 administration significantly potentiates chemotherapy antitumour activity either *in vitro* or *in vivo*, supporting the potential relevance of using ERK5 inhibitors in combination with current chemotherapy in breast cancer management [27,28].

In agreement, several other studies in breast cancer cell lines supported the relevance of ERK5 signalling in breast carcinogenesis. In fact, ERK5 activation leads to cell proliferation through modulation of CDKs and cyclin D1 [32,33], the epithelial–mesenchymal transition (EMT) [34,35], MET/HGF-induced migration [36], and integrin/FAK-mediated metastasis [37]. Remarkably, following knockdown of the MEK5/ERK5 pathway, tumour xenograft growth, metastasis, and the generation of tumour circulating cells was significantly reduced [35,38]. Nevertheless, in contrast with this body of data, a recent study reported that ERK5 expression was reduced in breast cancer tumour samples, correlating with a worse disease prognosis, and that ERK5 inhibition in MDA-MB-231 and Hs578 T breast cancer cell lines contributed to increased cell migration and invasion [39]. However, multiple additional studies demonstrated that ERK5 activation in MCF-7, MDA-MB-468, MDA-MB-453, and SKBR3 breast cancer cell lines promoted resistance to therapy, migration, and invasion [31,34,40,41], thus supporting ERK5 signalling as a potential therapeutic target in breast cancer. Moreover, increased active ERK5 was found in 70–80% of human breast tumours compared with adjacent tissue, and ERK5 was activated in all detected brain metastasis [31,42].

### *Prostate cancer*

Prostate cancer is the world's second-leading diagnosed cancer in men [23]. Current prostate cancer treatment includes prostatectomy and radiation therapy, sometimes supplemented with hormonal therapy or surgical removal of testicles. However, recurrence is common, and usually occurs in 15–40% of patients within 5–10 years of prostatectomy [43]. Although only a small

percentage of patients develop metastasis, metastatic prostate cancer almost invariably progresses to a hormone-independent or castrate-resistant state, with a median overall survival of approximately 15 months regardless of treatment applied [44]. Therefore, there is a demand for the development of novel therapeutic targets and biologically relevant biomarkers for the identification and treatment of malignant and metastatic forms of prostate cancer.

MEK5/ERK5 pathway deregulation in prostate cancer was first suggested in 1996 [45]. Later, both MEK5 and ERK5 were demonstrated to be overexpressed in human prostate cancer and, more importantly, implicated in prostate carcinogenesis [46,47]. In fact, ERK5 was found significantly upregulated in human prostate cancer metastasis, and correlated with more aggressive and metastatic disease and worse prognosis [44,46,47]. Consistently, increased ERK5 expression was also correlated with androgen-independent prostate cancer, and ERK5 nuclear localisation was associated with transition from hormone-dependent to hormone-independent disease [47]. Altogether, this body of clinical evidence suggests the importance of MEK5/ERK5 signalling in the acquisition of more aggressive tumour phenotypes. Furthermore, our understanding of the molecular mechanisms underlying MEK5/ERK5 signalling in prostate cancer has increased. In this regard, MEK5/ERK5 signalling was shown to promote prostate tumour cell proliferation by promoting the G1–S cell cycle phase transition [46,47] and induction of DNA replication [48]. Importantly, the relevance of ERK5 signalling over other MAPKs increased after observation that blocking ERK1/2 with PD184352 was insufficient to abrogate tumour cell proliferation; only when ERK5 was efficiently inhibited, using higher doses of PD184352, did tumour cells fail to proliferate [47]. Furthermore, MEK5/ERK5 signalling proved to be involved in PTKA-mediated migration [49], integrin/FAK-mediated metastasis [37], and matrix metalloproteinase (MMP)-1, -2 and -9 expression [44,46], thus suggesting the critical enrolment of ERK5 in prostate cancer metastasis and supporting the potential application of ERK5 inhibitors in metastatic prostate cancer management.

### Colon cancer

Colon cancer ranks as the third most-common cancer in men and second most-common cancer in women [23]. Currently, colonoscopy is the best-available screening test for early detection and timely resection of preneoplastic lesions. Improved therapeutics, although limited in efficacy, have been achieved with the implementation of 5-fluorouracil (5-FU) in colon cancer management, which, in combination with several systemic agents, has contributed to decreased colon cancer recurrence [50]. However, 20–25% of patients present with metastasis at the time of diagnosis, and 30–40% of patients will develop metastasis [51]. The metastatic and more aggressive form of the disease remains mostly incurable, being responsible for poor overall patient survival [52]. Therefore, the identification and development of novel relevant therapeutic targets is a priority in colon cancer.

The first evidence of MEK5/ERK5 signalling deregulation in colon cancer was indirectly provided by the observation that miRNA-143, whose main target is ERK5 mRNA, was typically downregulated in patients with colon cancer [53], suggesting the potentially increased expression of ERK5. Moreover, miRNA-143 downregulation is a typical tumour initiation event associated with accelerated tumorigenesis [53]. In agreement, others recently showed that the *MAPK7* gene is typically hypomethylated in primary colorectal cancer [54], suggesting the potential increased expression of ERK5. Supporting these data, it was demonstrated that MEK5 and ERK5 are overexpressed in human colon adenomas and adenocarcinomas, and that ERK5 upregulation significantly correlates with the acquisition of a more aggressive and metastatic tumour phenotype [55]. Consistently, phosphorylated MEK5, the active form of MEK5, was found upregulated in human colon cancer and correlated with advanced disease stages and with decreased overall survival of patients with colon cancer [56], thus supporting the involvement of MEK5/ERK5 signalling in colon carcinogenesis.

In addition, it was recently shown that increased activation of MEK5/ERK5 signalling in constitutively active MEK5 colon cancer cells contributed to the acceleration of cell cycle progression and increased tumour cell migration *in vitro*, and metastasis *in vivo*, when compared with colon cancer cells expressing dominant negative MEK5 (abrogated ERK5 activation) or to parental colon cancer cells [55]. In agreement with this study, others reported that ERK5 activation increased tumour cell growth where ERK5 repression was at least partially responsible for the inhibition of tumour cell migration and invasion, repressing the aggressive phenotype of colon cancer cells [57]. By contrast, it was recently reported that proliferation in colon cancer cells harbouring KRAS and BRAF mutations was not the result of ERK5 signalling, because ERK5 inhibition abrogated proliferation [58]. Notwithstanding, the authors also hypothesised a possible feed forward mechanism of ERK5 activation by ERK1/2 that could be a driver of innate resistance to MEK1/2 inhibitors in tumours harbouring KRAS and BRAF mutations [58].

It has previously been shown that forced expression of miRNA-143 in tumour xenografts, with concomitant repression of ERK5, reduced tumour growth and increased apoptosis [59]. In addition, miRNA-143 expression and repression of ERK5 hampered small intestine tumour development in APC $^{Min/+}$ mice [60]. Lastly, ERK5 inhibition by RNAi approaches or XMD8-92 proved to be a potential relevant therapeutic option in colon cancer management, contributing to significant chemosensitisation of colon cancer cells and xenografts to 5-FU treatment [61,62], and triggering natural killer (NK) cell recognition of colon tumour cells by the downregulation of major histocompatibility complex (MHC) class I expression [63]. Collectively, current knowledge supports the involvement of ERK5 signalling in colon carcinogenesis, where it has a role in the acquisition of a more aggressive and metastatic phenotype.

### Leukaemia

Leukaemia is characterised by the abnormal expansion of transformed progenitor white blood cells. These progenitor cells fail to differentiate into mature cells, including granulocytes or macrophages, and, as a result, accumulate inside bone marrow [64]. For the current treatment of leukaemia, particularly for acute myeloid leukaemia (AML) and acute lymphocytic leukaemia (ALL), two main strategies have been the focus of current research. These strategies aim to limit cell proliferation and promote either cell differentiation (differentiation therapies) or apoptosis of the

transformed progenitor cells (proapoptotic therapies) [64]. The importance of understanding the signalling circuitry in leukaemia arises from the inefficiency of treatment options in current treatment regimens that rarely lead to patient survival. In adults, most cases are incurable and the 5-year survival is 20%. In children, prognosis is better, although approximately 40% of paediatric patients will relapse [65]. Currently, a vitamin D3 derivative, $1\alpha,25(OH)_2$ vitamin D3 (1,25D), and its synthetic analogues, have been used to promote the differentiation of leukaemic cell lines, with promising antileukaemic action. Nevertheless, clinical trials with these compounds remain inconclusive [66].

The involvement of MEK5/ERK5 signalling in leukaemia still requires additional investigation because of the unclear role of ERK5 in differentiation therapies. Initial evidence reported that ERK5 mediates proliferation, and its activation is essential for the survival of leukaemic T cells. Importantly, the authors observed that leukaemic cells with stable ERK5 knockdown mediated by small hairpin (sh)ERK5 failed to develop tumours *in vivo* [67]. However, further studies have generated controversial and unclear results. Recently, ERK5 was shown to be involved in the monocytic differentiation of human AML cells upon cell treatment with 1,25D vitamin D3 [68]. In agreement with this study, others demonstrated that, in human AML cells, both established cultures and AML blasts *ex vivo*, ERK5 directly promoted the optimal monocytic differentiation of AML cells when exposed to differentiating agents [65]. However, in contrast with previous studies, it was also demonstrated that pharmacological inhibition of ERK5 signalling by XMD8-92 and BIX02189 in 1,25D-induced differentiating AML cells resulted in particularly robust cell cycle arrest at G2 phase and monocytic differentiation. Therefore, in this latter study, the use of ERK5 inhibitors in combination with vitamin D derivatives was suggested as a potential successful therapeutic approach in AML [66].

Several studies have argued in favour of the relevance of using ERK5 inhibitors in leukaemia treatment. This is highlighted by the fact that ERK5 is essential for the protection of reactive oxygen species (ROS)-induced apoptosis in ALL leukaemic cells [69], proliferation of AML cells [69,70], and resistance to therapeutics [71]. Furthermore, ERK5 inhibition blocked proliferation and induced apoptosis [10,69–71], increased cell death by NK cells as a consequence of decreased MHC-I expression [72], and sensitised leukaemic cells to cytarabine-induced apoptosis [73]. However, all these studies focused on the survival and apoptosis of cancer cells after ERK5 inhibition, rather than on its role in cell differentiation. Therefore, the role of ERK5 inhibition in myeloid or lymphocytic leukaemia treatment needs to be further explored to clarify its relevance as a target in leukaemia therapy, especially when associated with differentiation therapies.

### *Hepatocellular carcinoma*

Despite remarkable advances already achieved in the diagnosis and treatment of hepatocellular carcinoma, it still has the second-highest mortality rate [23,74–76]. Most hepatocellular carcinomas develop as a result of chronic liver disease, which, in combination with several other factors, such as inflammation, oxidative stress, and a hypoxic microenvironment, typically occurring in the liver, can have a role in tumour initiation, progression, and metastasis, importantly contributing to poorer prognosis and hampered therapeutics. Importantly, when hepatocellular carcinoma develops from cirrhosis, the use of surgery and other ablative techniques for tumour management is limited [74,75].

Knowledge regarding the role of ERK5 in the liver and in hepatocellular carcinoma is poor. Nevertheless, the first evidence for ERK5 deregulation in liver cancer was found after the discovery of *ERK5* gene amplification in human hepatocellular cell lines [74]. This was further confirmed and extended, demonstrating that the *ERK5* gene was amplified and ERK5 protein overexpressed in human hepatocellular cell lines and also in primary human hepatocellular carcinomas. Moreover, following ERK5 silencing, proliferation was significantly reduced and cells failed to undergo cell division [75]. Recently, in addition to the data reported thus far, ERK5 nuclear localisation was shown to be increased in hepatocellular carcinoma samples and in the neighbouring cirrhotic tissue from the same patient, when compared with normal liver tissue [76], suggesting its potential role in hepatocellular carcinoma initiation, particularly when developed from cirrhosis. Furthermore, ERK5 silencing or chemical inhibition by XMD8-92 was demonstrated to be a potential relevant therapeutic approach in hepatocellular carcinoma, after blocking tumour cell proliferation, preventing hypoxia-induced migration and invasion, and, more remarkably, abrogating tumour xenograft growth [76].

### *Osteosarcoma*

Osteosarcoma is an aggressive type of bone cancer generally detected in young people aged between 10 and 25 years, and whose 5-year overall survival is approximately 65–75%. However, patients with metastasis at the time of diagnosis, which represents 10–20% of patients with osteosarcoma, have a 5-year survival rate of <30%. Despite the dramatic increase in our understanding of sarcoma biology and treatment, the survival rate has not increased over the past two decades, with the main cause of patient death being pulmonary metastasis [77,78].

In 2002, the *MAPK7* gene was reported to be amplified in high-grade osteosarcoma [79]. However, only recently was ERK5 considered a potential therapeutic target. ERK5 overexpression correlated with tumour progression, resistance to treatment, and worse overall patient survival [78], suggesting that ERK5 has an important role in tumour development. However, in two additional studies, ERK5 silencing failed to decrease tumour cell proliferation as would be initially expected [77]. By contrast, ERK5 involvement in tumour metastasis seemed to be particularly more relevant because ERK5 silencing significantly influenced human osteosarcoma cell migration and invasion either *in vitro* or *in vivo*. In fact, ERK5 silencing abrogated the migration and metastasis potential of tumour cells in a mechanism dependent on Slug and MMP-9 [77]. Altogether, current knowledge regarding ERK5 signalling involvement in osteosarcoma is particularly relevant for the development of therapeutic strategies targeting the disseminated form of the disease.

### *Pancreatic cancer*

Pancreatic cancer currently has the worst overall patient survival, with a 5-year survival of <5% [23,80]. The usually asymptomatic, highly aggressive phenotype is the main reason for this high mortality. Invasion and metastasis occur at a very early stage of pancreatic cancer and most patients present with metastatic

disease at the time of diagnosis, making it impossible to surgically remove the tumour, although this remains the most effective therapeutic approach [80].

Thus far, the only evidence supporting the direct involvement of ERK5 in pancreatic cancer was provided by a recent study in which the authors observed that chemical inhibition of ERK5 with XMD8-92 significantly inhibited pancreatic cell tumour xenograft growth [81]. Beyond this study, most information regarding the potential involvement of ERK5 in pancreatic cancer arises from studies that report the involvement of miRNA-143 in pancreatic carcinogenesis. In this regard, several studies have already demonstrated that miRNA-143 is significantly deregulated in pancreatic cancer tissue and cell lines [82,83], although a solid consensus has not yet been achieved. On the one hand, some studies demonstrated that miRNA-143 is upregulated in pancreatic cancer tissue when compared with normal tissue [83], which is unexpected compared with other tumours of the gastrointestinal tract. On the other hand, other studies reported that miRNA-143 was downregulated in pancreatic cancer [82], especially in *KRAS* mutant pancreatic cancers, where restoration of miRNA-143 levels abrogated tumorigenesis [82]. This lack of consensus might result from the different criteria used to select pancreatic tumour samples. It is known that pancreatic cancer can often result from a chronic pancreatitis background. In fact, miRNA-143 levels were not differentially expressed between pancreatic cancer samples and chronic pancreatitis [83]. Although miRNA-143 was found to be upregulated in pancreatic tumour samples, miRNA-143 was negatively correlated with lymph node spreading, supporting its potential utility as a prognostic biomarker for the metastatic spread of pancreatic cancer [84]. Further evidence from additional recent studies demonstrated that miRNA-143-induced overexpression inhibited the proliferation, metastasis, and invasion of pancreatic cancer cells, and ultimately inhibited the growth of subcutaneous and orthotopic pancreatic cancer xenografts [85]. Given that the ERK5 transcript represents one of the most widely reported targets of miRNA-143, ERK5 could be related to the biological effects of miRNA-143 in pancreatic cancer, similarly to colon cancer, and could also represent a relevant target in this tumour type.

### *Lung cancer*

Lung cancer is the world's leading diagnosed cancer, and also leads the rank of cancer-related deaths [23]. The major risk factors for the development of lung cancer are tobacco smoke and predisposing genetic backgrounds, including EGFR, RAS or ALK mutations [86]. Aberrant activation of several effectors of the MAPK family, including ERK5, induced by tobacco smoke [87], and two typical EGFR mutants found in lung cancer [88], were the first two pieces of evidence suggesting the involvement of ERK5 in lung carcinogenesis. Moreover, the insulin-like growth factor (IGF)-II/ERK5/AMP response element binding protein (CREB) pathway was shown to have a vital role in lung tumorigenesis [89]. In addition, two consistent studies unveiled an atypical allele polymorphism in the ERK5 promoter and amplification of the *MAPK7* gene within in the Chinese population that led to enhanced ERK5 expression in lung tissue [90,91]. However, despite the evidence reported in these studies, the current state-of-the-art indicates that the role of ERK5 in this type of tumour remains unclear. In fact, peroxisome proliferator-activated receptor (PPAR)-γ activation via ERK5 represents a crucial event of the antitumourigenic effect of Wnt7a and Fzd9 in non-small cell lung carcinoma [92]. Furthermore, others observed that EMT triggered by tobacco smoke in lung tissue depends on ERK5 inhibition and, more remarkably, ERK5 inhibition by XMD8-92 mimics EMT induced by tobacco smoke in lung cancer cells [93]. In light of these results, ERK5 inhibition appears to be crucial in EMT, which was initially unexpected because tobacco smoke promotes oxidative stress [87], which in turn is known to activate ERK5 [12]. Intriguingly, beyond confirming the reduced ERK5 activity in lung cancer cell lines and lung tissue of animals exposed to tobacco smoke, it was recently observed that ERK5 activity was increased, rather than decreased, in the stomach, liver, kidney, and bladder tissue of animals exposed to tobacco smoke [93]. These results suggest that EMT regulation by ERK5 is tissue specific, and possibly also modulated by cell type and microenvironment specificities. Therefore, the use of ERK5 specific inhibitors in lung cancer treatment remains controversial because of its reported effects in EMT. Although requiring additional confirmatory studies, ERK5 inhibition in lung cancer in contrast to other tumours could promote progression to the metastatic form of the disease, rather than provide an antitumour effect. However, arguing in favour of ERK5 inhibition as a lung cancer strategy, others have observed that the chemical inhibition of ERK5 with XMD8-92 significantly reduced lung cancer xenograft growth, particularly in combination with the chemotherapeutic agent doxorubicin [94]. In addition, the expression of miRNA-143 has been reported to be significantly reduced in lung cancer [95] and, more importantly, the restoration of miRNA-143 levels reduced tumour growth and inhibited the invasion and metastasis of lung cancer [96]. However, none of these effects have been specifically related to ERK5 inhibition by miRNA-143, indicating that further studies are needed to precisely ascertain the potential of ERK5 targeting in lung cancer.

### *Other cancer types*

Although scarce, other lines of evidence suggest the involvement of ERK5 signalling in several other tumour types. In this regard, ERK5 activation has been recently demonstrated to be crucial in the ALK-induced transcription of MYCN and consequent promotion of aberrant proliferation of neuroblastoma cells. Interestingly, the inhibition of ERK5, by genetic or pharmacological approaches, enhanced the antitumour efficacy of crizotinib in both *in vitro* and *in vivo* models [97], supporting the therapeutic efficacy of targeting ERK5 in neuroblastoma. Moreover, ERK5 signalling pathway effectors also appear at the top of the list of deregulated molecules in bladder cancer [98], and miRNA-143 overexpression, with concomitant repression of ERK5, functions as a tumour suppressor in human bladder cancer [99]. Furthermore, ERK5 signalling has also been implicated in human mesothelioma cell proliferation, migration, invasion, and resistance to therapy [100] and, more importantly, has been suggested as a potential beneficial therapeutic target for the development of treatment strategies in patients with malignant mesothelioma [100]. Lastly, ERK5 overexpression has also been implicated in head and neck squamous carcinoma, and associated with advanced tumour stage and lymph node metastasis [101]. In fact, subsequent studies demonstrated that miRNA-143 upregulation led to the inhibition of cell migration and invasion [102], possibly related to ERK5 targeting.

All these scarce but consistent results lead to the preliminary conclusion that ERK5 signalling might also represent an interesting study topic in neuroblastoma, bladder cancer, mesotheliomas, and neck and head squamous cell carcinoma as a potential therapeutic target and/or prognostic marker.

Finally, and expanding the relevance of ERK5 as a therapeutic target in cancer, ERK5 was recently reported to be a crucial mediator of inflammation and inflammation-driven cancer [103]. Inflammation is a major risk factor for the development of several types of cancer, being recognised as an enabling cancer feature [104]. It is believed that the inflammatory microenvironment supports the survival, sustained proliferation of cells, and angiogenesis, facilitating either tumour initiation and/or the malignant transformation of cancer cells [103]. Although others have suggested the anti-inflammatory role of MAPKs, two recent studies demonstrated that ERK5 was able to control the expression of several specific inflammatory mediators in response to a range of toll-like receptor (TLR) agonist and proinflammatory cytokines, which are essential for the establishment of the inflammatory microenvironment and, for instance, needed to support skin carcinogenesis [103].

## Clinical relevance

The RAS-RAF-MEK-ERK signalling module, of which MAPK family members are part, is overactivated in several types of highly diagnosed cancer. Overactivation normally results from activating mutations of KRAS, NRAS, and BRAF, which lead to the downstream activation of MAPK [105]. Over the past decade, because of their earlier discovery, several MEK1/2 inhibitors have been developed and introduced in clinical setting. In single-agent therapeutic regimens, efficacy of these inhibitors is low, being more pronounced in tumours harbouring BRAF and NRAS mutations, where trametinib is a US Food and Drug Administration (FDA)-approved MEK inhibitor, indicated for the treatment of BRAF V600E/K mutation-positive unresectable or metastatic melanoma [105].

Possibly because of their later discovery, the development of MEK5 or ERK5 inhibitors and the translation of these inhibitors to the clinics have yet to be explored. MEK5/ERK5 inhibition has been revealed to have a significant influence on the sensitisation of cancer cells to chemotherapy agents, justifying the potential of MEK5/ERK5 inhibitors for future clinical use. Inhibition of ERK5 sensitised tumour cells to etoposide [40], trastuzumab [26], fulvestrant [31], tamoxifen [31], docetaxel [27,28], doxorubicin [28,94,100,103], cisplatin [27,100], vinorelbine [27], imatinib [106], dexamethasone [19], bortezomib [19], cytarabine [73], and crizotinib [97]. It has also been demonstrated that ERK5 inhibition, either through pharmacological or genetic approaches, leads to tumour cell sensitisation to 5-FU [61]. In light of these data, MEK5/ERK5 can be added to the list of MAPKs known to sensitise tumour cells to a range of chemotherapeutic drugs currently in clinical use (Table 1), warranting preclinical and clinical development of promising small-molecule inhibitors, such as XMD8-92 [107], used in combinatorial therapies in multiple cancer types. Of note, beyond all the evidence regarding ERK5 chemical inhibition reported and discussed in this review, the results obtained with XMD8-92 should now be treated with particular caution because of its recently discovered role in the direct inhibition of bromodomains [108]. Bromodomains correspond to conserved protein motifs that are responsible for the recognition of acetyl-lysine residues during transcriptional processes [108]. Although in several studies the results generated through XMD8-92 inhibition were similar to those obtain through specific ERK5-silencing techniques, new chemical inhibitors should be generated to avoid possible unspecific effects related to bromodomain inhibition.

**TABLE 1**

**ERK5 inhibition promotes chemotherapy sensitisation in several human tumours**

| Chemotherapy sensitisation | Tumour | *In vivo/in vitro* | ERK5 inhibition | Refs |
|---|---|---|---|---|
| **Etoposide** | Breast | *In vitro* | DN-MEK5 | [40] |
| **Trastuzumab** | Breast | *In vitro* | shERK5; $ERK5^{AEF}$ | [26] |
| **Fulvestrant** | Breast | *In vitro* | shERK5 | [31] |
| **Tamoxifen** | Breast | *In vitro* | shERK5 | [31] |
| **Docetaxel** | Breast | *In vivo + in vitro* | XMD8-92 | [28] |
| **Doxorubicin** | Breast | *In vivo + in vitro* | XMD8-92 | [28,94,100,103] |
| | Cervical carcinoma | *In vivo + in vitro* | XMD8-92 | |
| | Lung | *In vivo + in vitro* | XMD8-92 | |
| | Mesothelioma | *In vivo + in vitro* | shERK5 | |
| | Skin carcinoma | *In vivo + in vitro* | ERK5 $\Delta^{epidermis}$; XMD8-92 | |
| **Cisplatin** | Breast | *In vitro* | shERK5; TG02 | [27,100] |
| | Mesothelioma | *In vivo + in vitro* | shERK5 | |
| **Vinorelbine** | Breast | *In vitro* | shERK5; TG02 | [27] |
| **Imatinib** | CML (leukaemia) | *In vitro* | $ERK5^{AEF}$ | [106] |
| **Dexamethasone** | Multiple myeloma | *In vitro* | $ERK5^{AEF}$ | [19] |
| **Bortezomib** | Multiple myeloma | *In vitro* | $ERK5^{AEF}$ | [19] |
| **Cytarabine** | AML (leukaemia) | *In vitro* | siERK5 | [73] |
| **Crizotinib** | Neuroblastoma | *In vivo + in vitro* | siERK5; XMD8-92 | [97] |
| **5-FU** | Colon | *In vivo + in vitro* | siERK5; miR143; XMD8-92 | [61,62] |

[a]DN, dominant negative; $ERK5^{AEF}$, dominant negative form of ERK5; siERK5, ERK5 small interference RNA; Δ, knockout.

**TABLE 2**

**Clinical and molecular supporting evidence for ERK5 involvement in human tumour development and resistance**

| Evidence | Tumour | Refs |
|---|---|---|
| **Clinical evidence** | | |
| *Upregulation of ERK5 signalling in human carcinogenesis* | Breast | [26–28] |
| | Prostate | [46,47] |
| | Colon | [54–56] |
| | Osteosarcoma | [78,79] |
| | Hepatocellular carcinoma | [74–76] |
| | Lung | [90,91] |
| *Upregulation of ERK5 signalling correlates with poorer prognosis and/or acquired resistance to therapy* | Breast | [27–31] |
| | Prostate | [44–47] |
| | Colon | [56] |
| | Osteosarcoma | [78] |
| | Mesothelioma | [100] |
| *Upregulation of ERK5 signalling increases tumour metastasis incidence* | Breast | [31,32] |
| | Prostate | [44,46,47] |
| | Colon | [55,56] |
| | Osteosarcoma | [78] |
| | Mesothelioma | [100] |
| | HNSCC | [101] |
| **Molecular evidence** | | |
| *ERK5 signalling promotes tumour cell proliferation* | Breast | [32,33,35,38] |
| | Prostate | [46–48] |
| | Colon | [55,59] |
| | Hepatocellular carcinoma | [75,76] |
| | Pancreatic | [81] |
| | Neuroblastoma | [97] |
| | Mesothelioma | [100] |
| | Lung | [94] |
| | Leukaemia | [67,70,71] |
| | Bladder | [99] |
| *ERK5 signalling promotes tumour cell migration and metastasis* | Breast | [31–38,40,41] |
| | Prostate | [37,44,46,49] |
| | Colon | [55,57] |
| | Osteosarcoma | [77] |
| | Hepatocellular carcinoma | [76] |
| | Mesothelioma | [100] |

## Concluding remarks

Over the past few decades, the involvement of MAPK family members in cancer development has supported the potentially beneficial role of MAPK inhibitors alone or in combination with current cancer therapy, which has led to the incorporation of several MEK inhibitors in combination therapies, currently being tested in clinical trials [105]. Here, we have reviewed the rationale for MEK5/ERK5 targeting in tumour management and demonstrated that MEK5/ERK5 represents a potential relevant target in breast cancer, metastatic prostate cancer, colon cancer, and invasive osteosarcoma (Table 2). Although limited, current knowledge also suggests that MEK5/ERK5 represents a relevant prognostic factor or therapeutic target in hepatocellular carcinoma, pancreatic cancer, neuroblastoma, bladder cancer, mesothelioma, and head and neck squamous cell carcinoma (Table 2). Further evidence is required to assess the relevance of MEK5/ERK5 targeting in lung cancer and leukaemia therapy. Particularly in leukaemia, the inhibition of MEK5/ERK5 signalling is promising in the context of proapoptotic therapies, and its potential remains unclear in differentiation therapies.

In summary, patients with cancer and lower MEK5/ERK5 expression benefit from systemic treatment in contrast to those who present with higher levels of MEK5/ERK5 expression [29], with ERK5 inhibition inducing sensitisation to a range of antitumour agents. The possibility of targeting the MEK5/ERK5 pathway with small-molecule inhibitors, already in preclinical development, makes this pathway a desirable potential novel target in human cancer and paves the way for the short-term translation of MEK5 or ERK5 inhibitors into the clinic.

## References


1 Cargnello, M. and Roux, P.P. (2011) Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. *Microbiol. Mol. Biol. Rev.* 75, 50–83

2 Yang, S-H. *et al.* (2013) MAP kinase signalling cascades and transcriptional regulation. *Gene* 513, 1–13

3 Drew, B. *et al.* (2012) MEK5/ERK5 pathway: the first fifteen years. *Biochim. Biophys. Acta* 1825, 37–48

4 Zhou, G. *et al.* (1995) Components of a new human protein kinase signal transduction pathway. *J. Biol. Chem.* 270, 12665–12669

5 Lee, J.D. *et al.* (1995) Primary structure of BMK1: a new mammalian map kinase. *Biochem. Biophys. Res. Commun.* 213, 715–724
6 Lochhead, P.a *et al.* (2012) ERK5 and its role in tumour development. *Biochem. Soc. Trans.* 40, 251–256
7 Nithianandarajah-Jones, G.N. *et al.* (2012) ERK5: structure, regulation and function. *Cell. Signal.* 24, 2187–2196
8 Morimoto, H. *et al.* (2007) Activation of a C-terminal transcriptional activation domain of ERK5 by autophosphorylation. *J. Biol. Chem.* 282, 35449–35456
9 Iñesta-Vaquera, F.a. *et al.* (2010) Alternative ERK5 regulation by phosphorylation during the cell cycle. *Cell Signal.* 22, 1829–1837
10 Álvarez-Fernández, S. *et al.* (2013) Potent antimyeloma activity of a novel ERK5/CDK inhibitor. *Clin. Cancer Res.* 19, 2677–2687
11 Honda, T. *et al.* (2015) Phosphorylation of ERK5 on Thr732 is associated with ERK5 nuclear localization and ERK5-dependent transcription. *PLoS ONE* 10, e0117914
12 Abe, J. *et al.* (1996) Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. *J. Biol. Chem.* 271, 16586–16590
13 Kato, Y. *et al.* (1997) BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2 C. *EMBO J.* 16, 7054–7066
14 Kato, Y. *et al.* (1998) Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* 395, 713–716
15 Kesavan, K. *et al.* (2004) MEKK2 regulates the coordinate activation of ERK5 and JNK in response to FGF-2 in fibroblasts. *J. Cell. Physiol.* 199, 140–148
16 Hayashi, M. and Lee, J.D. (2004) Role of the BMK1/ERK5 signaling pathway: lessons from knockout mice. *J. Mol. Med.* 82, 800–808
17 Watson, F.L. *et al.* (2001) Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nat. Neurosci.* 4, 981–988
18 Nicol, R.L. *et al.* (2001) Activated MEK5 induces serial assembly of sarcomeres and eccentric cardiac hypertrophy. *EMBO J.* 20, 2757–2767
19 Carvajal-Vergara, X. *et al.* (2005) Multifunctional role of Erk5 in multiple myeloma. *Blood* 105, 4492–4499
20 Wang, X. and Tournier, C. (2006) Regulation of cellular functions by the ERK5 signalling pathway. *Cell. Signal.* 18, 753–760
21 Wang, X. *et al.* (2005) Targeted deletion of mek5 causes early embryonic death and defects in the extracellular signal-regulated kinase 5/myocyte enhancer factor 2 cell survival pathway. *Mol. Cell. Biochem.* 25, 336–345
22 Kimura, T.E. *et al.* (2010) Targeted deletion of the extracellular signal-regulated protein kinase 5 attenuates hypertrophic response and promotes pressure overload-induced apoptosis in the heart. *Circ. Res.* 106, 961–970
23 Ferlay, J. *et al.* (2013) *GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide*. IARC
24 Voduc, K.D. *et al.* (2010) Breast cancer subtypes and the risk of local and regional relapse. *J. Clin. Oncol.* 28, 1684–1691
25 Schnitt, S.J. (2010) Classification and prognosis of invasive breast cancer: from morphology to molecular taxonomy. *Mod. Pathol.* 23, S60–S64
26 Montero, J.C. *et al.* (2009) Expression of Erk5 in early stage breast cancer and association with disease free survival identifies this kinase as a potential therapeutic target. *PLoS ONE* 4, e5565
27 Ortiz-ruiz, M.J. *et al.* (2014) Therapeutic potential of ERK5 targeting in triple negative breast cancer. *Oncotarget* 5, 11308–11318
28 Al-Ejeh, F. *et al.* (2014) Kinome profiling reveals breast cancer heterogeneity and identifies targeted therapeutic opportunities for triple negative breast cancer. *Oncotarget* 5, 3145–3158
29 Miranda, M. *et al.* (2015) MEK5-ERK5 pathway associates with poor survival of breast cancer patients after systemic treatments. *Oncoscience* 2, 99–101
30 Polo, M.L. *et al.* (2010) Responsiveness to PI3 K and MEK inhibitors in breast cancer. Use of a 3D culture system to study pathways related to hormone independence in mice. *PLoS ONE* 5, e10786
31 Antoon, J.W. *et al.* (2013) MEK5/ERK5 signaling suppresses estrogen receptor expression and promotes hormone-independent tumorigenesis. *PLoS ONE* 8, e69291
32 Mulloy, R. *et al.* (2003) Activation of cyclin D1 expression by the ERK5 cascade. *Oncogene* 22, 5387–5398
33 Perez-Madrigal, D. *et al.* (2012) The extracellular-regulated protein kinase 5 (ERK5) promotes cell proliferation through the down-regulation of inhibitors of cyclin dependent protein kinases (CDKs). *Cell. Signal.* 24, 2360–2368
34 Zhou, C. *et al.* (2008) Proteomic analysis of tumor necrosis factor-alpha resistant human breast cancer cells reveals a MEK5/Erk5-mediated epithelial–mesenchymal transition phenotype. *Breast Cancer Res.* 10, R105
35 Javaid, S. *et al.* (2015) MAPK7 regulates EMT features and modulates the generation of CTCs. *Mol. Cancer Res.* 13, 934–944
36 Castro, N.E. and Lange, C.a. (2010) Breast tumor kinase and extracellular signal-regulated kinase 5 mediate Met receptor signaling to cell migration in breast cancer cells. *Breast Cancer Res.* 12, R60
37 Sawhney, R.S. *et al.* (2009) A novel role of ERK5 in integrin-mediated cell adhesion and motility in cancer cells via Fak signaling. *J. Cell. Physiol.* 219, 152–161
38 Cronan, M.R. *et al.* (2012) Defining MAP3 kinases required for MDA-MB-231 cell tumor growth and metastasis. *Oncogene* 31, 3889–3900
39 Zuo, Y. *et al.* (2015) Modulation of ERK5 is a novel mechanism by which Cdc42 regulates migration of breast cancer cells. *J. Cell. Biochem.* 116, 124–132
40 Weldon, C.B. *et al.* (2002) Identification of mitogen-activated protein kinase kinase as a chemoresistant pathway in MCF-7 cells by using gene expression microarray. *Surgery* 132, 293–301
41 Madak-Erdogan, Z. *et al.* (2014) Novel roles for ERK5 and cofilin as critical mediators linking ERα-driven transcription, actin reorganization, and invasiveness in breast cancer. *Mol. Cancer Res.* 12, 714–727
42 Da Silva, L. *et al.* (2010) HER3 and downstream pathways are involved in colonization of brain metastases from breast cancer. *Breast Cancer Res.* 12, R46
43 Fu, W. *et al.* (2012) Progress of molecular targeted therapies for prostate cancers. *Biochim. Biophys. Acta* 1825, 140–152
44 Ramsay, a.K. (2011) ERK5 signalling in prostate cancer promotes an invasive phenotype. *Br. J. Cancer* 104, 664–672
45 Robinson, D. *et al.* (1996) A tyrosine kinase profile of prostate carcinoma. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5958–5962
46 Mehta, P.B. *et al.* (2003) MEK5 overexpression is associated with metastatic prostate cancer, and stimulates proliferation, MMP-9 expression and invasion. *Oncogene* 22, 1381–1389
47 McCracken, S.R.C. *et al.* (2008) Aberrant expression of extracellular signal-regulated kinase 5 in human prostate cancer. *Oncogene* 27, 2978–3288
48 Dudderidge, T.J. *et al.* (2007) Mitogenic growth signalling, DNA replication licensing, and survival are linked in prostate cancer. *Br. J. Cancer* 96, 1384–1393
49 Zheng, Y. *et al.* (2012) Protein-tyrosine kinase 6 promotes peripheral adhesion complex formation and cell migration by phosphorylating p130 CRK-associated substrate. *J. Biol. Chem.* 287, 148–158
50 Dienstmann, R. *et al.* (2015) Personalizing colon cancer adjuvant therapy: selecting optimal treatments for individual patients. *J. Clin. Oncol.* 33, 1787–1796
51 Glynne-Jones, R. and Kronfli, M. (2011) Locally advanced rectal cancer: a comparison of management strategies. *Drugs* 71, 1153–1177
52 Wolpin, B.M. and Mayer, R.J. (2008) Systemic treatment of colorectal cancer. *Gastroenterology* 134, 1296–1310
53 Akao, Y. *et al.* (2006) MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol. Rep.* 16, 845–850
54 Tian, X. *et al.* (2015) Screening of potential diagnostic markers and therapeutic targets against colorectal cancer. *Onco. Targets. Ther.* 8, 1691–1699
55 Simões, A.E.S. *et al.* (2015) Aberrant MEK5/ERK5 signalling contributes to human colon cancer progression via NF-κB activation. *Cell Death Dis.* 6, e1718
56 Hu, B. *et al.* (2012) Expression of the phosphorylated MEK5 protein is associated with TNM staging of colorectal cancer. *BMC Cancer* 12, 127
57 Mansour, M. *et al.* (2015) SATB2 suppresses the progression of colorectal cancer cells via inactivation of MEK5/ERK5 signaling. *FEBS J.* 282, 1394–1405
58 Lochhead, P.A. *et al.* (2016) Tumor cells with *KRAS* or *BRAF* mutations or *ERK5/MAPK7* amplification are not addicted to ERK5 activity for cell proliferation. *Cell Cycle* 15, 506–518
59 Borralho, P.M. *et al.* (2011) miR-143 overexpression impairs growth of human colon carcinoma xenografts in mice with induction of apoptosis and inhibition of proliferation. *PLoS ONE* 6, e23787
60 Takaoka, Y. *et al.* (2012) Forced expression of miR-143 represses ERK5/c-Myc and p68/p72 signaling in concert with miR-145 in gut tumors of Apc(Min) mice. *PLoS ONE* 7, e42137
61 Borralho, P.M. *et al.* (2009) MicroRNA-143 reduces viability and increases sensitivity to 5-fluorouracil in HCT116 human colorectal cancer cells. *FEBS J.* 276, 6689–6700
62 Pereira, D.M. *et al.* (2016) MEK5/ERK5 signaling inhibition increases colon cancer cell sensitivity to 5-fluorouracil through a p53-dependent mechanism. *Oncotarget* Published online April 29, 2016. http://dx.doi.org/10.18632/oncotarget.9107
63 Sun, S. *et al.* (2014) Inhibition of protein carbamylation in urea solution using ammonium containing buffers. *Anal. Biochem.* 446, 76–81
64 Manzotti, G. *et al.* (2015) Monocyte-macrophage differentiation of acute myeloid leukemia cell lines by small molecules identified through interrogation of the Connectivity Map database. *Cell Cycle* 14 2578-2578
65 Wang, X. *et al.* (2014) ERK5 pathway regulates transcription factors important for monocytic differentiation of human myeloid leukemia cells. *J. Cell. Physiol.* 229, 856–867
66 Wang, X. *et al.* (2014) ERK 5/MAPK pathway has a major role in 1α,25-(OH)2 vitamin D3-induced terminal differentiation of myeloid leukemia cells. *J. Steroid Biochem. Mol. Biol.* 144, 223–227

67 Garaude, J. *et al.* (2006) ERK5 activates NF-kappaB in leukemic T cells and is essential for their growth *in vivo*. *J. Immunol.* 177, 7607–7617
68 Zheng, R. *et al.* (2014) 1,25-Dihydroxyvitamin D3 induces monocytic differentiation of human myeloid leukemia cells by regulating C/EBPβ expression through MEF2 C. *J. Steroid Biochem. Mol. Biol.* 5, 1–6
69 Lopez-Royuela, N. *et al.* (2014) Extracellular-signal-regulated kinase 5 modulates the antioxidant response by transcriptionally controlling Sirtuin 1 expression in leukemic cells. *Int. J. Biochem. Cell Biol.* 53, 253–261
70 Razumovskaya, E. *et al.* (2011) Inhibition of MEK5 by BIX02188 induces apoptosis in cells expressing the oncogenic mutant FLT3-ITD. *Biochem. Biophys. Res. Commun.* 412, 307–312
71 Suzuki, T. and Yang, J. (2014) Hydrogen peroxide activation of ERK5 confers resistance to Jurkat cells against apoptosis induced by the extrinsic pathway. *Biochem. Biophys. Res. Commun.* 444, 248–253
72 Charni, S. *et al.* (2009) ERK5 knockdown generates mouse leukemia cells with low MHC class I levels that activate NK cells and block tumorigenesis. *J. Immunol.* 182, 3398–3405
73 Xu, Y. *et al.* (2015) Inhibition of ERK5 enhances cytarabine-induced apoptosis in acute myeloid leukemia cells. *Int. J. Clin. Exp. Med.* 8, 6446–6455
74 Liu, L. *et al.* (2003) Gene expression profiles of hepatoma cell line HLE. *World J. Gastroenterol.* 9, 683–687
75 Zen, K. *et al.* (2009) ERK5 is a target for gene amplification at 17p11 and promotes cell growth in hepatocellular carcinoma by regulating mitotic entry. *Genes Chromosom. Cancer* 48, 109–120
76 Rovida, E. *et al.* (2014) The mitogen-activated protein kinase ERK5 regulates the development and growth of hepatocellular carcinoma. *Gut* 64, 1–12
77 Yue, B. *et al.* (2014) ERK5 silencing inhibits invasion of human osteosarcoma cell via modulating the Slug/MMP-9 pathway. *Eur. Rev. Med. Pharmacol. Sci.* 18, 2640–2647
78 Tesser-Gamba, F. *et al.* (2012) MAPK7 and MAP2K4 as prognostic markers in osteosarcoma. *Hum. Pathol.* 43, 994–1002
79 van Dartel, M. *et al.* (2002) Amplification of 17p11.2 approximately p12, including PMP22, TOP3A, and MAPK7, in high-grade osteosarcoma. *Cancer Genet. Cytogenet.* 139, 91–96
80 Singh, P. *et al.* (2011) Major molecular markers in pancreatic ductal adenocarcinoma and their roles in screening, diagnosis, prognosis, and treatment. *Pancreas* 40, 644–652
81 Sureban, S.M. *et al.* (2014) XMD8-92 inhibits pancreatic tumor xenograft growth via a DCLK1-dependent mechanism. *Cancer Lett.* 351, 151–161
82 Kent, O.A. *et al.* (2010) Repression of the miR-143/145 cluster by oncogenic Ras initiates a tumor-promoting feed-forward pathway. *Genes Dev.* 24, 2754–2759
83 Bloomston, M. *et al.* (2007) MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. *JAMA* 297, 1901–1908
84 Tavano, F. *et al.* (2012) Changes in miR-143 and miR-21 expression and clinicopathological correlations in pancreatic cancers. *Pancreas* 41, 1280–1284
85 Pramanik, D. *et al.* (2011) Restitution of tumor suppressor microRNAs using a systemic nanovector inhibits pancreatic cancer growth in mice. *Mol. Cancer Ther.* 10, 1470–1480
86 Mitsudomi, T. (2014) Molecular epidemiology of lung cancer and geographic variations with special reference to EGFR mutations. *Transl. Lung Cancer Res.* 3, 205–211
87 Zhong, C.Y. *et al.* (2005) MAPK/AP-1 signal pathway in tobacco smoke-induced cell proliferation and squamous metaplasia in the lungs of rats. *Carcinogenesis* 26, 2187–2195
88 Jiang, J. *et al.* (2005) Epidermal growth factor-independent transformation of Ba/F3 cells with cancer-derived epidermal growth factor receptor mutants induces gefitinib-sensitive cell cycle progression. *Cancer Res.* 65, 8968–8974
89 Linnerth, N.M. *et al.* (2005) IGF-II induces CREB phosphorylation and cell survival in human lung cancer cells. *Oncogene* 24, 7310–7319
90 Qiu, F. *et al.* (2013) A functional polymorphism in the promoter of ERK5 gene interacts with tobacco smoking to increase the risk of lung cancer in Chinese populations. *Mutagenesis* 28, 561–567
91 Gavine, P.R. *et al.* (2015) Identification and validation of dysregulated MAPK7 (ERK5) as a novel oncogenic target in squamous cell lung and esophageal carcinoma. *BMC Cancer* 15, 454
92 Winn, R.a. *et al.* (2006) Antitumorigenic effect of Wnt 7a and Fzd 9 in non-small cell lung cancer cells is mediated through ERK-5-dependent activation of peroxisome proliferator-activated receptor gamma. *J. Biol. Chem.* 281, 26943–26950
93 Liang, Z. *et al.* (2015) ERK5 negatively regulates tobacco smoke-induced pulmonary epithelial–mesenchymal transition. *Oncotarget* 2, 15–21
94 Yang, Q. *et al.* (2013) BMK1 is involved in the regulation of p53 through disrupting the PML-MDM2 interactions. *Oncogene* 32, 3156–3164
95 Gao, W. *et al.* (2010) Deregulated expression of miR-21, miR-143 and miR-181a in non small cell lung cancer is related to clinicopathologic characteristics or patient prognosis. *Biomed. Pharmacother.* 64, 399–408
96 Xia, H. *et al.* (2014) miR-143 inhibits NSCLC cell growth and metastasis by targeting Limk1. *Int. J. Mol. Sci.* 15, 11973–11983
97 Umapathy, G. *et al.* (2014) The kinase ALK stimulates the kinase ERK5 to promote the expression of the oncogene MYCN in neuroblastoma. *Sci. Signal.* 7, ra102
98 Lambrou, G. *et al.* (2013) Gene expression is highly correlated on the chromosome level in urinary bladder cancer. *Cell Cycle* 12, 1544–1559
99 Noguchi, S. *et al.* (2011) MicroRNA-143 functions as a tumor suppressor in human bladder cancer T24 cells. *Cancer Lett.* 307, 211–220
100 Shukla, A. *et al.* (2013) Extracellular signal-regulated kinase 5: a potential therapeutic target for malignant mesotheliomas. *Clin.Cancer Res* 19, 2071–2083
101 Sticht, C. *et al.* (2008) Activation of MAP kinase signaling through ERK5 but not ERK1 expression is associated with lymph node metastases in oral squamous cell carcinoma (OSCC). *Neoplasia* 10, 462–470
102 Ni, Z.Y. *et al.* (2015) Decreased microRNA-143 expression and its tumor suppressive function in human oral squamous cell carcinoma. *Genet. Mol. Res.* 14, 6943–6952
103 Finegan, K.G. *et al.* (2015) ERK5 is a critical mediator of inflammation-driven cancer. *Cancer Res.* 75, 742–753
104 Hanahan, D. and Weinberg, R.a. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674
105 Zhao, Y. and Adjei, A.a. (2014) The clinical development of MEK inhibitors. *Nat. Rev. Clin. Oncol.* 11, 385–400
106 Buschbeck, M. *et al.* (2005) Abl-kinase-sensitive levels of ERK5 and its intrinsic basal activity contribute to leukaemia cell survival. *EMBO Rep.* 6, 63–69
107 Yang, Q. *et al.* (2010) Pharmacological inhibition of BMK1 suppresses tumor growth through promyelocytic leukemia protein. *Cancer Cell* 18, 258–267
108 Lin, E.C.K. *et al.* (2016) ERK5 kinase activity is not required for cellular immune response. *bioRxiv* http://dx.doi.org/10.1101/038513 (epub ahead printing)