

Review

# A Special View of What Was Almost Forgotten: p38 $\delta$ MAPK

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**Simple Summary:** p38 $\delta$  MAPK, as well as the other p38 isoforms, was proposed as a drug target for cancer therapy, owing to its critical roles in cellular signaling. However, herein, we show that p38 $\delta$  inhibition may be therapeutically beneficial for treatment of specific cancer types, such as skin carcinoma, hepatocellular carcinoma (HCC), head and neck squamous cell carcinoma (HNSCC), cholangiocarcinoma (CC), and breast cancer. This review aims to discuss the roles of p38 $\delta$  in cancer and summarize the findings of molecules with potential to inhibit p38 $\delta$  in order to guide the search for new target cancer therapies based on p38 $\delta$  inhibitors.

**Abstract:** The p38 $\delta$  mitogen-activated protein kinase is an important signal transduction enzyme. p38 $\delta$  has recently emerged as a drug target due to its tissue-specific expression patterns and its critical roles in regulation of cellular processes related to cancer and inflammatory diseases, such as cell proliferation, cell migration, apoptosis, and inflammatory responses. However, potent and specific p38 $\delta$  inhibitors have not been defined so far. Moreover, in cancer disease, p38 $\delta$  appears to act as a tumor suppressor or tumor promoter according to cancer and cell type studied. In this review, we outline the current understanding of p38 $\delta$  roles in each cancer type, to define whether it is possible to delineate new cancer therapies based on small-molecule p38 $\delta$  inhibitors. We also highlight recent advances made in the design of molecules with potential to inhibit p38 isoforms and discuss structural approaches to guide the search for p38 $\delta$  inhibitors.

**Keywords:** MAPK13; p38 $\delta$  MAPK; p38 delta; SAPK4; cancer; p38 $\delta$  inhibitors



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## 1. Introduction

Mitogen-activated protein kinase (MAPK) cascades are signaling components that play a key role in transducing extracellular stimuli into cellular responses through phosphorylation of different substrates. MAPK signaling pathways control a variety of cellular processes, such as growth, differentiation, proliferation, survival, and death [1,2]. The mammalian family of MAPKs includes the extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase (JNK), and p38. Each of these cascades has at least three components: a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK), and a MAPK, which are phosphorylated and activated in sequence [3].

The p38 MAPK signaling pathways are activated by a wide variety of environmental and cellular stresses—for example, oxidative stress, hypoxia, and UV irradiation—or by inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1 $\beta$  [4,5]. The upstream proteins MKK3 and MKK6 are responsible for activation of p38 MAPK [6]. MKK4 can also phosphorylate p38; however, this upstream protein is also an activator

of JNK [7]. Once phosphorylated, p38 activates diverse cytoplasmic or nuclear protein substrates by catalyzing the transfer of the terminal  $\gamma$ -phosphate from the ATP to them. Substrates can be transcription factors, protein kinases, cytoskeletal proteins, and other cellular targets [5].

Four members comprise the p38 MAPK family: p38 $\alpha$  (MAPK14), p38 $\beta$  (MAPK11), p38 $\gamma$  (MAPK12 or stress-activated protein kinases 3 - SAPK3), and p38 $\delta$  (MAPK13 or SAPK4). Based on their amino acid sequence, different expression patterns, and sensitivity to chemical inhibitors, p38 isoforms can be separated into two subgroups: p38 $\alpha$  and p38 $\beta$  represent one subgroup with 75% sequence identity, while p38 $\gamma$  and p38 $\delta$  represent the other subgroup (62% and 61% sequence identity with p38 $\alpha$ , respectively) [8]. While p38 $\alpha$  is ubiquitously and abundantly expressed in cell lines and tissues, p38 $\beta$  is expressed at very low levels (compared to p38 $\alpha$ ), and its function seems to be redundant with p38 $\alpha$  [9]. In contrast, p38 $\gamma$  and p38 $\delta$  have a tissue-specific expression—for example, p38 $\gamma$  in skeletal muscle and p38 $\delta$  in endocrine glands [4,5]. Each subgroup of p38 isoforms also has specific substrates: MAPK-activated protein kinase-2 and 3 (MK2 and MK3) are examples of substrates preferentially phosphorylated by p38 $\alpha$  and p38 $\beta$  isoforms, while microtubule-associated protein Tau appears to be a better substrate for p38 $\gamma$  and p38 $\delta$  [4,10,11]. Altogether, these observations suggest different cellular functions for each p38 isoform.

Due to the involvement of p38 MAPKs in cell transformation processes, such as unlimited proliferation, protection against apoptotic cell death, angiogenesis, tissue invasion, and metastasis, p38 isoforms emerged as drug targets for cancer therapy [2,12,13]. For a long time, p38 $\alpha$  was the best-characterized isoform and the focus of numerous drug design projects. However, this has changed over time. p38 $\alpha$  knockout mice die at the embryonic stage due to placental morphological defects, while p38 $\beta$ , p38 $\gamma$ , and p38 $\delta$  knockout mice are viable and lack developmental defects [14–17]. Furthermore, mice lacking both p38 $\gamma$  and p38 $\delta$  demonstrated no detrimental phenotypes and decreased innate inflammatory responses induced by lipopolysaccharide (LPS) [18]. Therefore, p38 $\gamma$  and p38 $\delta$  isoforms may be more viable targets than p38 $\alpha$ .

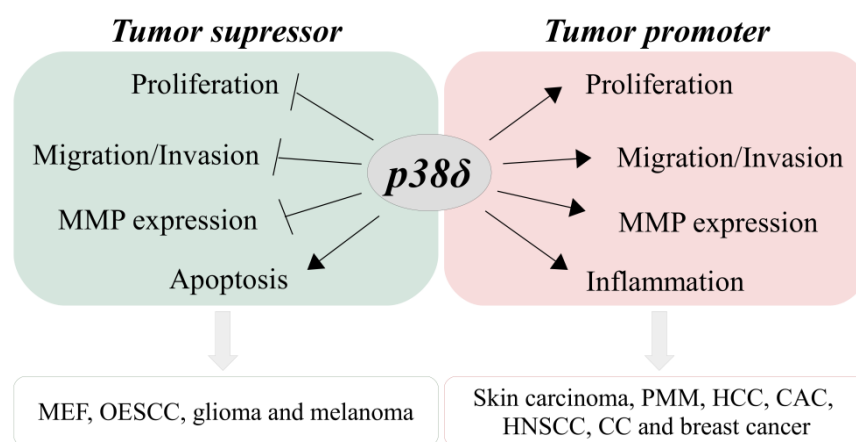
Although most scientific attention used to be focused on p38 $\alpha$ , the p38 $\delta$  isoform has recently emerged as a potential drug target due to its involvement in the regulation of cellular processes such as stress response, cytokines production, proliferation, migration, differentiation, and apoptosis [18–20]. In contrast to p38 $\alpha$ , p38 $\delta$  has distinct expression patterns depending on the tissue and, consequently, specific biological effects. Variable levels of p38 $\delta$  expression were detected in endocrine tissues, including pituitary, prostate, salivary, and adrenal glands, and in pancreas, small intestine, testis, colon, kidney, and lung [10,21,22].

Physiological roles for p38 $\delta$  have been reported in human disorders, such as neurodegenerative diseases [23], diabetes [17], inflammatory diseases [19,24,25], and cancer [26–30]. In cancer, p38 $\delta$  appears to have contrasting roles: tumor suppressor or tumor promoter, which can change according to cancer and cell type studied [28,29,31–33]. Acting as tumor promoter, p38 $\delta$  seems to also have a role in cancer invasion and metastasis [26,27]. Although these contrasting p38 $\delta$  functions have been described, the specific roles played by p38 $\delta$  on each cancer type have not been precisely defined.

This review aims to outline the current understanding of p38 $\delta$  MAPK roles in cancer, for the purpose of determining the specific function of this enzyme in each cancer type and in tumor development. The restricted expression patterns and specialized functions of p38 $\delta$  in cancer development, metabolic, and inflammatory diseases opens the possibility to delineate new target therapies based on small-molecule p38 $\delta$  inhibitors. Although the design of p38 inhibitors is ongoing, p38 $\delta$  specific inhibitors have not been discovered [34,35]. Therefore, this review also highlights recent efforts made to design molecules with potential to inhibit p38 $\delta$ , summarizing findings and structural information on p38 family members to guide the search for specific p38 $\delta$  inhibitors.

## 2. p38 $\delta$ MAPK in Cancer-Suppressor or Promoter?

The p38 $\delta$  MAPK is activated in response to extracellular stimulus, such as cellular stresses and inflammatory cytokines, through dual phosphorylation on threonine (Thr180) and tyrosine (Tyr182) residues by the upstream kinases MKK6 or MKK3 [4,36]. However, it has been shown that MKK3 is the main activator of p38 $\delta$  in fibroblasts cells that were exposed to cellular stresses, including hyperosmotic shock, ultraviolet radiation, and TNF- $\alpha$  cytokine, while MKK6 did not affect p38 $\delta$  activation under these stimuli [6]. Activation of p38 $\delta$  leads to phosphorylation of different substrates and, consequently, to several cellular responses. Some of these responses are associated with malignant transformation and cancer disorders, including cell proliferation, invasion, migration, and apoptosis [20,37]. However, p38 $\delta$  appears to have both tumor suppressor and tumor promoter roles, depending on the cancer and cell type studied (Figure 1).



**Figure 1.** The contrasting roles of p38 $\delta$  in cancer. MMP (matrix metalloproteinase), MEF (mouse embryonic fibroblasts), OESCC (oesophageal squamous cell carcinoma), PMM (pleural malignant mesothelioma), HCC, CAC (colitis-associated colorectal cancer), HNSCC, and CC.

The first indication of p38 $\delta$  as a tumor suppressor was observed in MEF. In these cells, p38 $\delta$  appears to regulate processes that protect cells against malignant transformation, such as control of cell density, cell–cell contact inhibition, and cell migration [31]. p38 $\delta$ <sup>-/-</sup> MEFs, but not p38 $\gamma$ <sup>-/-</sup> and wild-type (WT) MEFs, changed their morphology after reaching confluence and presented impaired contact inhibition. Moreover, either p38 $\delta$ <sup>-/-</sup> or p38 $\gamma$ <sup>-/-</sup> MEFs proliferated faster than WT fibroblasts and displayed increased cell migration and matrix metalloproteinase 2 (MMP2) secretion [31]. Overexpression of MMPs in cells lacking p38 $\delta$  was also observed in the human glioma-derived cell line SF767. In these cells, silencing of p38 $\delta$  increased secretion of MMP2 and MMP9 and also promoted cell invasion [38]. MMPs are enzymes responsible for degrading several components of the extracellular matrix, and several studies associate their high expression levels with angiogenesis, cell motility, and metastatic spread of cancer [39,40]. Thus, the evidence that p38 $\delta$  plays a role in the control of cell migration, invasion, and MMPs expression suggests that this enzyme is important for preventing cell transformation processes and decrease tumor progression.

The p38 $\delta$  roles in control of cell proliferation and migration were also observed in oesophageal squamous cell carcinoma (OESCC). OESCC cell lines lacking p38 $\delta$  expression proliferate faster than those endogenously expressing this isoform [32]. Nonetheless, reintroduction of p38 $\delta$  into OESCC cells lacking p38 $\delta$  expression resulted in the reduction of cell proliferation, migration, and number of colonies formed on soft agar, compared to WT cells [32]. Moreover, OESCC cells expressing p38 $\delta$  are more sensitive to a combination chemotherapy than p38 $\delta$ -deficient cells [41]. Therefore, in OESCC, p38 $\delta$  appears to not only control proliferation but also regulates apoptosis.

A study revealed that epigenetic alterations may be responsible for suppression of p38 $\delta$  expression in OESCC and, consequently, for tumor growth [42]. In OESCC cell lines, no mutations were identified in the MAPK13 gene sequence (encoding p38 $\delta$ ); however, hypermethylation of the MAPK13 promoter was found and associated with loss of p38 $\delta$  expression [42]. Epigenetic alterations of the MAPK13 gene were also found in primary cutaneous melanoma, associated with tumor growth [43]. Approximately 67% of primary melanoma and 85% of metastatic melanoma presented promoter methylation of the MAPK13 gene, associated with downregulation of p38 $\delta$  mRNA and protein expression [43]. Re-expression of p38 $\delta$  in melanoma cells with MAPK13 promoter methylation reduced proliferation, indicating that p38 $\delta$  may act as a tumor suppressor in melanoma cell lines [43]. Alternatively, the hypermethylation of MAPK13 gene promoter might, therefore, be an epigenetic cause of tumorigenesis promotion in cancer types where p38 $\delta$  has antioncogenic roles.

In contrast to its tumor suppressor roles, p38 $\delta$  also has tumor promoter roles, as evidenced in several types of cancers, such as skin carcinoma [29,44,45], pleural malignant mesothelioma (PMM) [46], HCC [47], colitis-associated colorectal cancer (CAC) [33], head and HNSCC [28,30], CC [26], and breast cancer [27]. The potential tumor promoter roles of p38 $\delta$  are summarized in Figure 1.

Studies with the two-stage 7,12-dimethylbenz(a) anthracene/12- O-tetradecanoylphorbol-13-acetate (DMBA/TPA) chemical carcinogenesis model have evidenced a tumor promoter role for p38 $\delta$  in skin squamous cell carcinoma (SCC). Specifically, p38 $\delta$ -deficient mice were resistant to development DMBA/TPA-induced skin carcinoma, with a significant delay in tumor growth [44]. Moreover, both tumor number and size were significantly decreased in p38 $\delta$  knockout mice compared to WT mice. This tumor reduction was associated with decreased levels of pro-proliferative ERK1/2-AP1 pathway and decreased activation of signal transducer and activator of transcription 3 (Stat3) [44]. Since Stat3 is an oncogenic transcription factor that plays a role in malignant conversion of skin tumors and is important for the phases of tumor initiation and promotion, p38 $\delta$  might promote skin carcinogenesis (in part) through the activation of Stat3 [44,48]. The tumor promoter role of p38 $\delta$  in DMBA/TPA induced skin tumorigenesis was confirmed by Zur and coworkers [45]. In mice, loss of p38 $\gamma$ , p38 $\delta$ , or both reduced DMBA/TPA-induced skin tumor development [45].

Recently, mice lacking p38 $\delta$  in epidermal keratinocytes or myeloid (immune) cells were employed to uncover the *in vivo* functional roles for p38 $\delta$  in promotion of DMBA/TPA chemical skin carcinogenesis. Keratinocyte-specific p38 $\delta$  ablation on mice did not influence the incidence or multiplicity of chemically induced skin tumors but reduced malignant progression in male and female mice in comparison to WT [29]. However, only in female mice, the tumors' volume was increased during the TPA promotion stage, demonstrating a sex-specific mechanism of tumor development [29]. In contrast, in myeloid cells, intrinsic p38 $\delta$  may be essential for the malignant progression of DMBA/TPA-induced skin tumors in males [29]. Myeloid cell-specific p38 $\delta$  loss in male mice inhibited DMBA/TPA-induced skin tumorigenesis during both promotion and malignant progression stages, with reduced tumor incidence, multiplicity, and volume, compared to WT [29]. These evidences support that p38 $\delta$  has tumor promoter roles in SCC and regulates skin carcinogenesis with different functions depending on the cellular context and stage of the tumor.

Additionally, p38 $\delta$  has been reported as tumor promoter in pleural malignant mesothelioma (PMM), a rare and dangerous tumor mainly occurring in the pleural space of the lung. Three p38 MAPK isoforms are expressed by rat PMM cells, but only the p38 $\delta$  isoform mediates proliferation stimulating signals [46]. In rat PMM cells stimulated with platelet-derived growth factor (PDGF)-BB, the induced proliferation was suppressed by downregulation of p38 $\delta$ , evidencing the pro-proliferative role of p38 $\delta$  [46]. The pro-oncogenic role of p38 $\delta$  was also confirmed in gynecological cancer stem-like cells (CSCs)/cancer-initiating cells (CICs). CSCs/CICs are small subpopulations of tumor cells that are endowed with higher self-renewal, tumor-initiating, and differentiation abilities, becoming resistant to treat-

ments [49,50]. CSCs/CICs isolated as aldehyde dehydrogenase (ALDH) high population, by RT-PCR, derived from HEC-1 cells (human endometrial carcinoma cells), MCAS and HTBoA cells (human ovarian cells), preferentially expressed the genes MAPK13 (p38 $\delta$ ), PTTG1IP, CAPN, and UBQLN2 over ALDH<sup>low</sup> cells [51]. MAPK13 gene knockdown using siRNA reduced the ALDH<sup>high</sup> population and abrogated tumor-initiating ability [51].

Another indication for a specific role of p38 $\delta$  in promoting cancer progression has recently been demonstrated in HCC. This study identified a multikinase inhibitor, compound AD80, with p38 $\gamma$  and p38 $\delta$  as its targets and antitumoral activity across a variety of HCC in vivo and in vitro models [47]. Moreover, growth inhibition assays and long-term clonogenic assays on HCC cell lines revealed that AD80 was more potent in these cells than Sorafenib, a compound commonly used in HCC therapy [47]. The potential pro-oncogenic role of p38 $\gamma$  and p38 $\delta$  on aggressive HCC was confirmed by analysis of The Cancer Genome Atlas (TCGA) liver cancer dataset. Significantly better survival was correlated with low mRNA expression levels of p38 $\gamma$  and p38 $\delta$  in HCC patient samples [47].

In some types of cancer, p38 $\delta$  appears to promote carcinogenesis by regulating chronic inflammation, one of the hallmarks associated with tumor development and progression [18,20,33,52]. The tumor microenvironment is composed not only by cancer cells but also by tumor-promoting inflammatory cells that can release growth factors, chemokines, and cytokines responsible for tumor development [52]. Since formation of an inflammatory microenvironment is an essential component of all tumors, the expression profile of cytokines and chemokines can be indicative of tumor progression [53]. Some studies showed that both p38 $\delta$  and p38 $\gamma$  modulate cytokine and chemokine production, immune cell migration, and T-cell activation [18,25,33,54,55]. For example, in LPS-stimulated macrophages from p38 $\gamma$ / $\delta$ -deficient mice, production of cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-10 are severely reduced, whereas IL-12 and IFN $\beta$  production increases [18].

The increased release of inflammatory cytokines and chemokines by p38 $\delta$  may enable formation of a proinflammatory microenvironment that sustains survival and growth of cancer cells. However, this inflammatory response is not exclusively activated by p38 $\delta$  pathway but also relay on strong contribution of p38 $\gamma$ . In CAC, a colon cancer subtype associated with inflammatory bowel disease, p38 $\delta$  and p38 $\gamma$  promoted colon tumorigenesis through regulation of inflammatory responses [33]. When compared to WT controls, mice deficient in p38 $\gamma$  and p38 $\delta$  exhibited reduced number of colon tumors, which correlated with a decrease in cytokines (IL-1 $\beta$  and TNF- $\alpha$ ), chemokines production (MCP1, KC, and MIP-2), and in inflammatory cell infiltration in colon [33]. The same was observed in a TPA-induced skin tumor model, where p38 $\gamma$ / $\delta$ <sup>-/-</sup> mice, in response to TPA, decrease the production of proinflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, and decrease production of the chemokines KC and MIP-2, resulting in decreased neutrophil recruitment to skin [45]. p38 $\delta$  was also associated with induction of IL-6 formation in human breast adenocarcinoma cell line MCF-7 [56].

Overexpression of the cytokines TNF- $\alpha$ , IL-1, and IL-6, which are enhanced by p38 $\delta$ , has been associated with induction of angiogenesis, invasion, and metastasis of cancer cells [57–59]. Metastasis is a complex process that involves a series of steps. At some point in carcinogenesis, cells can lose cell–cell and cell–matrix contacts, detach from the primary tumor, and invade adjacent tissues. Through lymphatic or blood vessels, cells can spread to distant organs, where they can proliferate and create new colonies of cancer cells [52,60]. In addition to the tumor promoter role described in events of cell proliferation and tumor growth, p38 $\delta$  has also been linked to activation of invasion and metastasis, another important hallmark of cancer [52]. p38 $\delta$  seems to be responsible for loss of contact inhibition, cell invasion, cell migration, and increased metastatic lesions [26,27], which are important for detachment of primary tumor cells and, consequently, their growth in distant metastasis.

The first study attributing a role to p38 $\delta$  in invasive migration of cancer cells was carried out on HNSCC. Inhibition of endogenous p38 $\delta$  or p38 $\alpha$  activity, the highly expressed p38 isoforms in HNSCC cell lines, effectively reduced invasion of HNSCC cells

through collagen [30]. The inhibition of these isoforms also reduced mRNA expression of matrix metalloproteinases 1 and 13 (MPP1 and MPP13) [30]. Overexpression of MPP1 and MPP13 were correlated with increase of tumor aggressiveness and disease poor prognosis, due to their roles in the degradation of extracellular matrix, angiogenesis, and metastatic cascade [61–64]. Recently, all four p38 MAPK isoforms were found highly elevated in serum of HNSCC patients, compared to the control group; however, only p38 $\alpha$ , p38 $\beta$ , and p38 $\delta$  serum levels were decreased after therapy in follow-up patients [28].

The roles of p38 $\delta$  in the promotion of cell migration and invasiveness were subsequently confirmed in CC [26]. p38 $\delta$  expression was found to be upregulated in CC tissues when compared with cancer adjacent normal liver tissues and normal biliary tract tissues [26]. Knockdown of p38 $\delta$  expression by siRNA transfection significantly inhibited motility of CC cells lines. Moreover, the knockdown of p38 $\delta$  also led to a decrease in motility and invasiveness of CC cells, in a wound-healing assay and Matrigel invasion assay, respectively [26]. On the other hand, overexpression of p38 $\delta$  increased invasiveness of CC cells lines, suggesting that p38 $\delta$  might be related to CC metastasis [26].

p38 $\delta$  has also been demonstrated to promote growth, migration, invasion, and metastasis of breast cancer by increasing cell proliferation and cell detachment. Comparison of p38 $\delta$  protein expression in human breast cancer tissue with noncancer tissues revealed that, in normal tissues, p38 $\delta$  was limited to ductal epithelium, while in breast cancer tissues, invasive ductal carcinoma sections had p38 $\delta$  increased [27]. Likewise, in mouse breast cancer model MMTV-PyMT, p38 $\delta$  was enhanced in breast tumor and lung tissue metastasis, compared to WT mice [27]. Loss of p38 $\delta$  in MMTV-PyMT mice reduced tumor volume and, beyond 14 weeks, promoted a reduction in lung metastatic lesion exceeding 90% [27]. On the other hand, knockdown of p38 $\delta$  in human breast cancer cell lines MCF-7 and MDA-MB-231 reduced cell proliferation in both cell lines and protected from detachment MDA-MB-231 cells stimulated with phorbol 12-myristate 13-acetate (PMA). Moreover, MDA-MB-231 cells lacking p38 $\delta$  decreased cellular migration in a scratch-wound-healing assay [27]. These studies evidence that p38 $\delta$  may not only regulate and affect growth of primary tumor but also promote the development of metastasis in breast cancer. Thus, p38 $\delta$  is a promising target for treatment of early and advanced stages of breast cancer.

An association between activation of the p38 $\delta$  pathway with tumor metastasis and growth was also observed in gastric cancer. The expression levels of gene G antigen 7B (GAGE7B), a member of the GAGE family that is involved in immune response, was found to be upregulated in poorly differentiated gastric cancer cell lines and in samples from advanced stage cancers (stages III-IV), compared to a well-differentiated cell line and samples from earlier stages (I-II) of gastric cancer, respectively [65]. Furthermore, GAGE7B was increased in metastatic tissues compared to nonmetastatic [65]. Gastric cancer cell lines overexpressing GAGE7B enhanced cell invasion, angiogenesis, and distant metastatic ability, and increased protein expression levels of p38 $\delta$ , pMAPKAPK2, and pHSP27. In addition, the authors found that in gastric cancer samples with lymph node metastasis the miR-30c, a miRNA, was downregulated, while GAGE7B was upregulated. Overexpression of miR-30c in two gastric cancer cells suppressed both GAGE7B and p38 $\delta$  expression, as well as inhibiting the migration and invasion ability of these cells, suggesting that the GAGE7B-induced p38 $\delta$  activity might be related to gastric cancer metastasis [65]. Although some studies demonstrated that p38 MAPK signaling can activate HSP27 through MAPKAPK2 phosphorylation [66,67], there is no evidence that this occurs specifically by p38 $\delta$  isoform. Therefore, the possibility that GAGE7B promotes tumor metastasis and growth of gastric cancer via activation of the p38 $\delta$ /pMAPKAPK2/pHSP27 pathway requires further studies to be confirmed.

The majority of cancer-associated deaths are caused by metastatic disease rather than primary tumors [68]. Given the tumor promoter roles of p38 $\delta$ , including cell proliferation, invasion, migration, and metastasis, this p38 isoform has emerged as a cancer drug target. The interest in targeted therapies with p38 $\delta$  inhibitors is justifiable since the loss of this enzyme could not only prevent growth of primary tumor but also prevent the disease

from progressing to an advanced stage with reduction of cell metastatic ability. Improving knowledge on this “forgotten” p38 isoform is an important advance toward treatment of some cancer types, especially for skin carcinoma, hepatocellular carcinoma, head and neck squamous cell carcinoma, cholangiocarcinoma, and breast cancer. Thus, for the types of cancers that p38 $\delta$  has tumor promoting roles, it is possible to delineate new cancer therapies based on small-molecule p38 $\delta$  inhibitors.

### 3. Searching Specific p38 $\delta$ MAPK Inhibitors

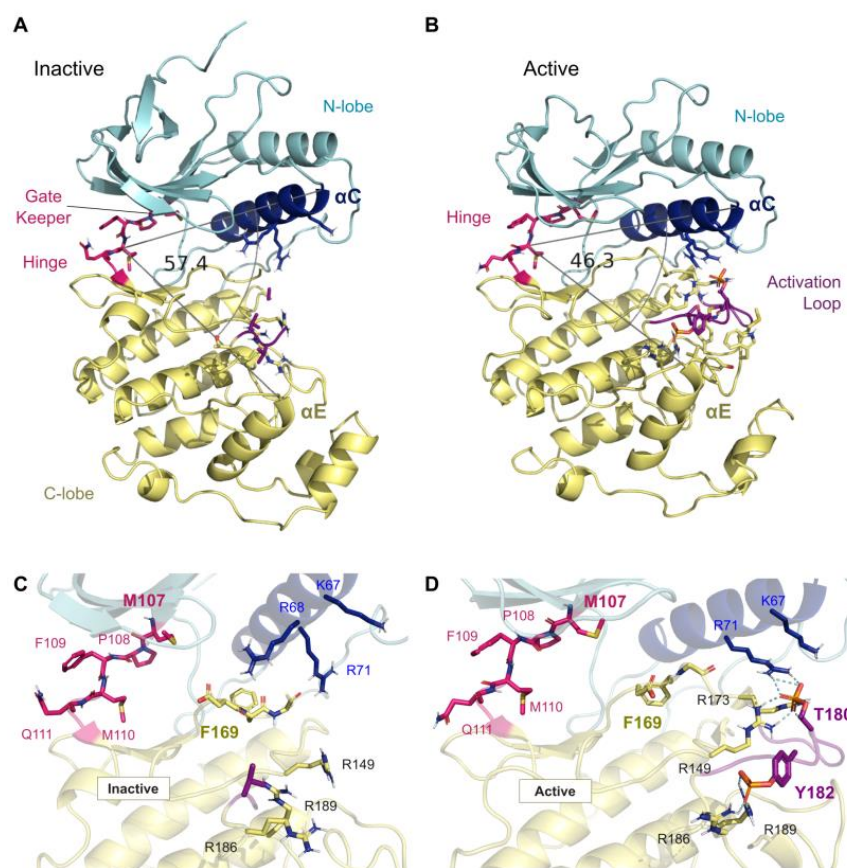
Small-molecule inhibitors have become a valuable tool for the development of targeted therapies, since these compounds can be highly selective and effective against specific proteins. Several studies have been carried out with small-molecule inhibitors targeting protein kinases that control proliferation and death of cancer cells [34,69–71]. Therefore, these special molecules may potentially be more selective and less aggressive for cancer therapy than other compounds. At least 20 p38 inhibitor candidates are in clinical trials, some of which for the treatment of cancer [13,35]. Nonetheless, to date, there are no approved compounds with potential to inhibit p38 and several p38 $\alpha$  inhibitors have failed in drug trials due to the high toxicity. Despite recent studies on isoform p38 $\gamma/\delta$ -selective inhibitors for the treatment of cutaneous T-cell lymphoma (CTCL), none of the candidates in clinical trials focus specifically on the inhibition of the p38 $\delta$  isoform (as reviewed by [35]).

The highly conserved ATP-binding site of the catalytic kinase domain is the major target of small-molecule kinase inhibitors. Like other protein kinases, p38 isoforms share a high degree of structural similarity within the ATP binding site, creating a challenge for designing selective inhibitors targeting this site [8,72]. Although p38 inhibitors differ on chemical structure and how they interact with the protein, most of them inhibit the enzyme by preventing ATP binding [72]. The challenge, therefore, relies on developing small-molecule inhibitors specific against p38 $\delta$ , with minimal off-targets interactions, in order to decrease the chances of toxicity in later clinical trials. In order to explore less-conserved areas and improve inhibitor selectivity, it is necessary to know in detail the structure of p38 isoforms, particularly p38 $\delta$ , as well as possible structural approaches that may guide this search.

As mentioned, all protein kinases share a similar structure; p38 isoforms, like other kinases, are folded into two subdomains: (i) an N-terminal subdomain (N-lobe) consisting of a five-stranded  $\beta$ -sheet and a long  $\alpha$ -helix ( $\alpha$ C-helix) and (ii) a large C-terminal subdomain (C-lobe) composed of six  $\alpha$ -helices [8,73]. The cleft between the lobes forms the ATP-binding site. These two lobes are connected by a flexible hinge region that facilitates the movement between them, as well as being involved in ATP binding [73,74].

All p38 MAPKs exhibit two structural states: active and inactive, according to their phosphorylation state. The region that regulates p38 activity is the activation loop, where the conserved amino acids threonine (Thr) and tyrosine (Tyr) (TGY-motif) are localized [8,73]. Dual phosphorylation of the Thr and Tyr residues in p38 structures by an upstream kinase stabilizes the activation loop in an open conformation, increasing affinity for ATP and facilitating substrate binding [75,76]. Therefore, this dual phosphorylation locks the enzyme in an active conformation.

The structure of p38 $\delta$ , either in the active and inactive state, displays the standard structure of protein kinases, consisting of N- and C-lobes with a catalytic site between them [8] (Figure 2A,B). However, in the active form, the overall p38 $\delta$  structure and catalytic site are more compact owing to interactions of phosphorylated Thr and Tyr with residues that coordinate the phosphorylation [8]. Although there is a high degree of sequence similarity between p38 isoforms, they rely on different residues to stabilize the negative charge introduced by the dual phosphorylation and to stabilize the active state (Figure 2C,D). Specifically, in p38 $\delta$ , phosphorylation of residues Thr180 and Tyr182 is coordinated by R71, R173, and R186, which are similar in p38 $\alpha$  and p38 $\gamma$ , as well as R149 and R189, which are only similar in p38 $\gamma$  [8]. Inhibitors that impair this coordination would likely stabilize the p38 $\delta$  inactive conformation [8].



**Figure 2.** Overview of the p38 $\delta$  in the (A) inactive (PDB ID 4YNO) and (B) active states (PDB ID 4MYG). Highlighting the N- and C-lobes in cyan and yellow, respectively, the hinge region in pink, activation loop in purple, and the alpha-C helix in dark blue. It is noteworthy that the active state has a tighter conformation with a smaller angle between the alpha-C and E helices. Highlights of the ATP-binding of both the (C) inactive and (D) active, with the double phosphorylation, states. Depicting the DFG motif into the inwards conformation (DFG-in) and inactive (DFG-out), respectively. The gate-keeper residue (Met107) is depicted as sticks. In the DFG-out state, Phe169 moves out of the pocket and expose a new site which type-II inhibitors can explore and bind. The Phe169 (F169) side chain is shown in both figures. Structure p38 $\delta$  DFG-in (PDB ID 4EYM); structure p38 $\delta$  DFG-out. Image generated in PyMOL.

The design of specific p38 inhibitors could explore different structural elements of protein kinases to introduce potency and selectivity, since these enzymes are dynamic and can adopt multiple conformations [73]. An example is represented by the highly conserved motif Asp-Phe-Gly (DFG-motif) localized in the C-lobe of kinases that forms part of the ATP-binding site. According to the position of the DFG-motif, protein kinases can assume two major conformations: DFG-in and DFG-out, which can determine protein active or inactive state, respectively [73,77]. In the DFG-in conformation, protein kinases typically assume a structure where the Phe169 residue of the DFG-motif is packed into a hydrophobic pocket in the groove between the N- and C-lobes, forming a cleft where substrates can bind [76,78]. In addition, the Asp168 residue of the DFG-motif is at the beginning of the activation loop, where it can coordinate a magnesium ion bind that interacts with an oxygen atom of the  $\beta$ -phosphate of ATP [73,76]. A DFG-in conformation was already observed in the p38 $\delta$  structure. When p38 $\delta$  is activated by dual phosphorylation, the side chain of Asp168 rotates, which prevents the movement of Phe169 side chain out of the hydrophobic pocket [8] (Figure 2A).

On the other hand, in the DFG-out conformation, Asp168 flips and Phe169 moves out of the DFG-pocket [77]. This DFG-flip induces structural changes in the activation loop of

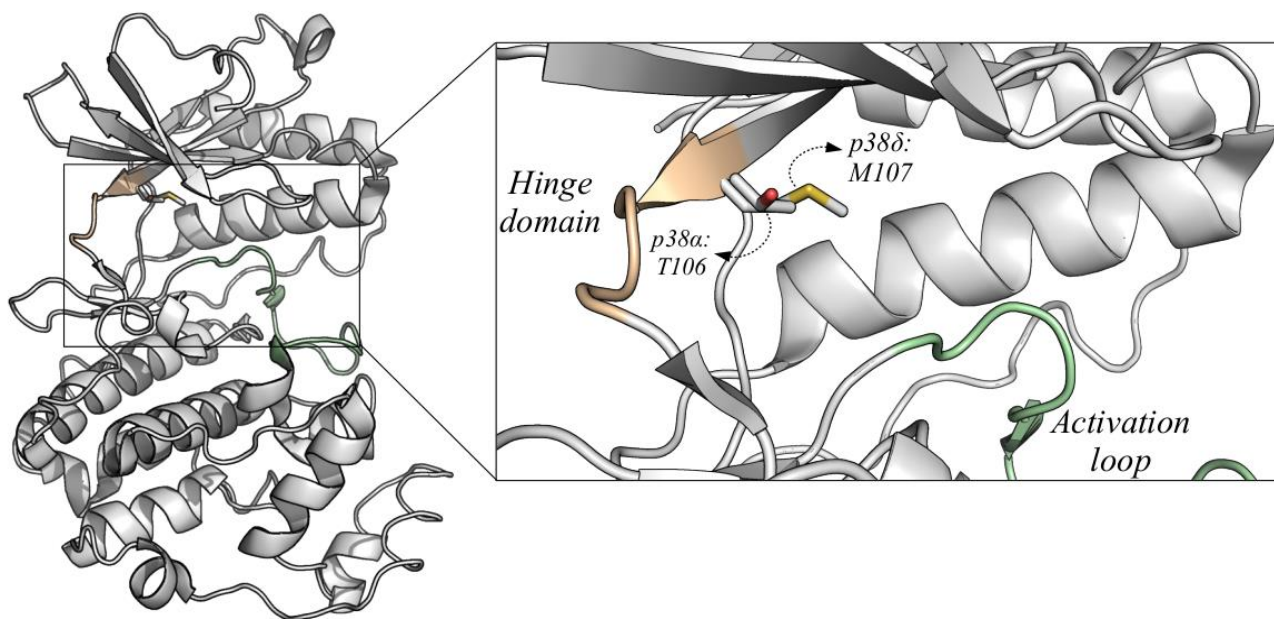


the enzyme, obstructing part of the ATP binding site and exposing an additional hydrophobic site in the kinase [47,79]. The position of DFG-motif in DFG-out is incompatible with ATP binding; therefore, the protein is at the inactive state [79]. Furthermore, in DFG-out (Figure 2C), the unoccupied DFG-pocket becomes more accessible for inhibitor binding.

p38 MAPK inhibitors were designed following the DFG-out and DFG-in binding mode. According to their mechanisms of inhibition, p38 inhibitors can be classified into three main categories: type-I, type-II, and type-1 $\frac{1}{2}$  [34,35]. Type-I inhibitors are ATP-competitive compounds that occupy only the adenine-binding pocket, forming from one to three hydrogen bonds with the backbone residues of the hinge region [78,80]. These ligands typically bind in a DFG-in conformation, recognizing the active state. The compounds SB203580 and Skepinone-L are good examples of highly potent inhibitors that bind to p38 $\alpha$  in DFG-in mode [81,82]. In contrast, inhibitors defined as type-II bind in the DFG-out state and occupy, in addition to the ATP-binding site, the new hydrophobic site accessible in the inactive form due to the rotation of DFG-motif [78]. Type-II inhibitors bind to the ATP site through hydrogen bonds with the hinge region and also exploit hydrogen bonding and hydrophobic interactions within the hydrophobic site, stabilizing the inactive state [83]. The compound BIRB796 is an example of type-II inhibitor of p38 that binds in the DFG-out mode of the enzyme and indirectly competes with ATP [79].

The type-1 $\frac{1}{2}$  is a subtype of type-I, since inhibitors of this class bind to the ATP-binding site like type-I compounds. However, these ligands also extend into a mainly hydrophobic back cavity of the ATP site where they make specific interactions similar to type-II inhibitors, typical of the DFG-out state [78]. Therefore, type-1 $\frac{1}{2}$  inhibitors are capable of binding the kinase in the activated and inactivated states. The back cavity that type-1 $\frac{1}{2}$  ligands explore is not occupied by the ATP co-substrate, and its size depends on the gatekeeper residue, an important residue of kinases which is located in the first position of the hinge region [78]. Kinases with gatekeeper residues with bulky side chains (e.g., Met, Phe, or Leu) have a small back cavity that reduces the space that inhibitors could explore, while small gatekeeper residues (e.g., Thr or Val) increase the size of the cavity [78]. An improvement in selectivity and potency of p38 $\alpha$  MAPK inhibitors was observed when they explored this new binding mode [84,85]. Nonetheless, to date, there has been no type-1 $\frac{1}{2}$  inhibitor described for p38 $\delta$ .

The potency and selectivity of p38 inhibitors may be better in the p38 isoforms with smaller gatekeeper residues. Instead of threonine (Thr) present in p38 $\alpha$  and p38 $\beta$ , p38 $\delta$  and p38 $\gamma$  possess as gatekeeper residue a bulky methionine (Met), which might interfere in the binding of some types of compounds (Figure 3). For example, p38 $\alpha$  and p38 $\beta$  isoforms are susceptible to inhibition by pyridinyl imidazoles, such as compounds SB203580 and SB202190, while p38 $\delta$  and p38 $\gamma$  are insensitive [10,86,87]. A single amino acid substitution in the gatekeeper residue in p38 $\delta$  and p38 $\gamma$ , replacing Met by Thr, makes them sensitive to SB203580 [87]. Kondoh and coworkers described an approach to guide a primary screening for selective inhibitors against p38 $\delta$  and p38 $\gamma$  based on this gatekeeper residue variation [86]. The approach consists of a comparative screening using chemical arrays aiming to identify specific inhibitors that specifically bind to p38 $\alpha$ -T106M mutant, which mimics the p38 $\delta$ /p38 $\gamma$  gate-keeper configuration, and not to p38 $\alpha$  wild-type [86]. The chemical arrays used in the study contained thousands of compounds and two fluorescent markers, and these arrays were treated with GST-p38 $\alpha$  wild-type or GST-p38 $\alpha$ <sup>T106M</sup> mutant. Since the probe had two markers, specific binders of GST-p38 $\alpha$ <sup>T106M</sup> were detected as red spots, while specific binders of GST-p38 $\alpha$  were green [86]. Therefore, it was possible to detect compounds that bind specifically to p38 $\delta$  and p38 $\gamma$ , as these proteins naturally have a Met as the gatekeeper residue.

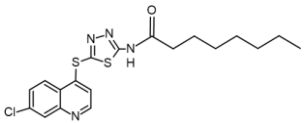
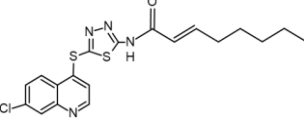
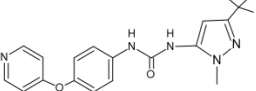
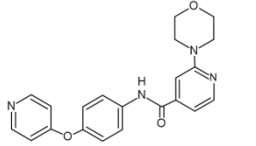
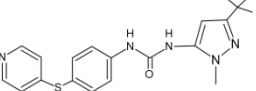
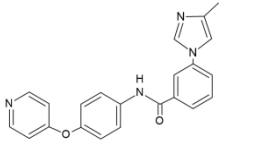
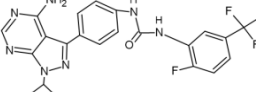
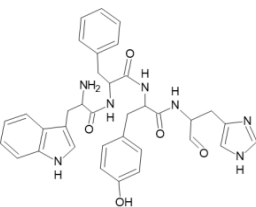


**Figure 3.** Structure of p38 $\delta$  and the gatekeeper residue. Ribbon diagram of p38 $\delta$  (PDB 4myg) showing the hinge domain in orange and the activation loop in green, regions near the ATP binding pocket. The p38 $\alpha$  gatekeeper residue (threonine, T106) is shown in the structure of p38 $\delta$  to compare with the p38 $\delta$  gatekeeper residue (methionine, M107). Methionine is a bulky residue and takes up more space, which might impair the binding and selectivity of inhibitors. Therefore, it is important to consider this difference for design of p38 $\delta$  inhibitors. Image generated in PyMOL.

The chemical array described to screening selective p38 $\delta$  and p38 $\gamma$  inhibitors identified the compound SU-002 as an inhibitor of p38 $\alpha$ <sup>T106M</sup> mutant, and in vitro kinases assays confirmed the potential of SU-002 to preferentially inhibit p38 isoforms with gatekeeper residue Met over Thr [86]. SU-002 is a compound with a 2-(7-chloroquinolin-4-yl) sulfanyl-1,3,4-thiadiazole (CQT) structure that has attachment of a long alkyl chain (octanamide) (Table 1). To improve this favorable structure to inhibit p38 $\delta$  and p38 $\gamma$ , the compound SU-005 was synthesized changing the octanamide of SU-002 by an oct-2-enamide attached to CQT structure (Table 1) [86]. SU-005 specifically decreased p38 $\delta$  and p38 $\gamma$  activity both in vitro and in cells lines and did not inhibit p38 $\delta$  with a Met107Thr mutant, suggesting that this compound may be a specific inhibitor of p38 $\delta$  and p38 $\gamma$  owing to the presence of the gatekeeper residue Met [86]. The authors also suggested that an inhibitor structure with an octanamide or oct-2-enamide attached to the CQT structure is responsible for targeting the Met gatekeeper residue and, consequently, preferentially inhibits p38 $\delta$  and p38 $\gamma$ .

The gatekeeper methionine of p38 $\delta$  may also be responsible for its poor sensitivity to the type-II inhibitor BIRB796, since this compound has a naphthalene group that sterically clashes with this large residue in p38 $\delta$  structure [19]. Aiming to obtain better p38 $\delta$  inhibitors, three compounds—61, 62, and 124—analogs of BIRB796 have been designed, replacing its naphthalene group to a smaller monoaryl ring (Table 1) [19]. Two of them, compounds 61 and 62, presented the expected binding in the DFG-out state of p38 $\delta$  and highly inhibited p38 $\delta$  activity. On the other hand, compound 124 adopted a DFG-in binding mode and showed lower inhibitory activity against p38 $\delta$ . However, all the three designed compounds also inhibited the activity of p38 $\alpha$ , which shows that the changes made in their structures were not sufficient to interfere with the binding to other p38 isoforms [19]. Although the compounds were not specific to p38 $\delta$ , they are a step forward in the knowledge about what type of inhibitors will inhibit with higher potency to p38 $\delta$ . While BIRB796 had an IC<sub>50</sub> of 1968 nM against p38 $\delta$ , compounds 61 and 62 presented IC<sub>50</sub> values of 620 nM and 281 nM, respectively [19]. Thus, it appears that potent p38 $\delta$  inhibitors can be designed following the DFG-out binding mode, with structures that do not clash with the Met gatekeeper residue in the hinge region of p38 $\delta$ .

**Table 1.** Structures of potential p38 $\delta$  inhibitors.

Name	Structure	Binding Mode <sup>1</sup>	Reference
SU-002		-	[86]
SU-005		-	[86]
61		DFG-out	[19]
124		DFG-in	[19]
58		DFG-out	[88]
117		DFG-in	[88]
AD80		DFG-out	[47]
WFYH		-	[28]

<sup>1</sup> Compounds without binding mode is because it was not specified in the study.

Yurtsever and coworkers also observed that compounds with binding in the DFG-out conformation of p38 $\delta$  had higher potency than those with binding in the DFG-in conformation [88]. The authors characterized two new p38 $\delta$  inhibitors, compound 58 and 117, and compared them to compounds previously described, 61 and 124 (Table 1). Similar to compound 61, compound 58 showed a DFG-out binding mode and IC<sub>50</sub> in the nanomolar range. In contrast, compound 117 displayed a DFG-in binding mode (similar to compound 124) and IC<sub>50</sub> in the micromolar range [88]. Moreover, the DFG-out inhibitors (compounds 58 and 61) displayed slower dissociation rates as compared to DFG-in inhibitors (compounds 117 and 124), which may lead to improvement in inhibitor activities [88].

A screening for type-II kinase inhibitors in a panel of five models of HCC cell lines was performed to find potential treatments for hepatocarcinoma. The study identified AD80 as a highly potent compound in HCC cell lines compared to Sorafenib based on growth inhibition and clonogenic assays [47]. AD80 also proved to be more potent than Sorafenib in a tumor xenograft model, with higher animal survival and increase in reduction of tumor growth rate per day [47]. Proteomic screening and biochemical analysis revealed that p38 $\delta$  and p38 $\gamma$  were the direct targets of AD80 [47]. Interestingly, the gatekeeper

residue appears to control AD80 selectivity to both isoforms of p38, and it seems to prefer enzymes with larger gatekeeper residues. Point mutations in p38 $\alpha$  and p38 $\beta$  replacing Thr106 for Met were performed, as well as substitution of Met107 to Thr in p38 $\delta$  (p38 $\delta^{M107T}$ ) and Met109 to Thr in p38 $\gamma$  (p38 $\gamma^{M109T}$ ). Biochemical melting temperature assays revealed that AD80 binds more favorably in p38 $\alpha^{T106M}$  and p38 $\beta^{T106M}$  mutants than in p38 $\delta^{M107T}$  and p38 $\gamma^{M109T}$  mutants [47].

A recent study developed a peptide inhibitor against p38 $\delta$  with the proposal to be used in the treatment of HNSCC, named WFYH (Table 1) [28]. Virtual screening was performed with a tetrapeptide library and p38 $\delta$  as the target protein, and ten tetrapeptides were selected for postdocking analysis. The peptide WFYH showed the best docking scores and good binding affinity for p38 $\delta$  in contrast to the other p38 isoforms [28]. WFYH at 75  $\mu$ M inhibited 77% of p38 $\delta$  activity, based on ELISA assays using activating transcription factor 2 (ATF-2) as a substrate [28]. The authors suggested that this peptide may have greater binding affinity for p38 $\delta$  over the other p38 isoforms owing to the strong noncovalent  $\pi$ - $\pi$  interaction that the benzene ring of Trp of WFYH made with Phe169 residue (in DFG-motif) of p38 $\delta$  and that this interaction also prevents Phe169 from moving.

An approach involving targeting protein kinases upstream of p38 $\delta$  MAPK could be an alternative strategy to achieve specific inhibition of p38 $\delta$  [89]. The upstream MKK3 is known to be a specific activator of all p38 MAPK isoforms. Nonetheless, in colorectal cancer (CRC) cell lines, MKK3 has been identified as a specific activator of the p38 $\delta$  isoform [90]. MKK3 was found highly expressed in the late stage of CRC, and its overexpression was correlated to poor prognosis [90]. Moreover, in CRC lines, MKK3 silencing increased the efficacy of the chemotherapeutic 5-FU, an effect that was associated with inhibition of p38 $\delta$  [90]. It was also observed that when CRC lines were exposed to 5-FU, p38 $\delta$  phosphorylation and activation were induced by MKK3; therefore, this molecular signaling may be responsible for decreasing the efficacy of the molecule [90]. p38 $\delta$  knockdown in CRC lines also demonstrated anti-proliferative effects; however, as presently, p38 $\delta$  specific inhibitors do not exist, it has been proposed that MKK3 inhibitors may be a potential treatment for colorectal cancer owing to the inhibition of MKK3/p38 $\delta$  pro-survival signaling [89,90]. The benefit of MKK3 inhibitors over p38 inhibitors lacking specificity can be based on the fact that, in CRC, while p38 $\delta$  has tumor promoter roles, p38 $\alpha$  seems to be a tumor suppressor [89]. Thereby, the selectivity of p38 inhibitors is crucial for cancer therapeutic purposes.

#### 4. Conclusions

p38 $\delta$  MAPK isoform for a long time was practically forgotten or did not arouse the interest of researchers, mostly because it is not an abundant protein expressed in all tissues as p38 $\alpha$ . However, as demonstrated in this review, p38 $\delta$  has some advantages as a drug target due to its restricted expression pattern. Furthermore, p38 $\delta$  has several important biological roles, especially in cancer disease where it seems to be a tumor suppressor or promoter, depending on the cancer type. There are several evidences showing that p38 $\delta$  is responsible for proliferation, migration, and invasion of tumor cells, which is critical for tumor progression and formation of metastasis. Therefore, p38 $\delta$  inhibition is a potential therapeutic strategy for the treatment of cancer, not only for those in the early stage, but also for advanced stages of the disease. In this context, it is important to take into account that the contrasting roles of p38 $\delta$  could bring some prejudicial responses when this isoform is inhibited, since it can stop tumor progression in a tissue, while leading to a malignant transformation in others. The selectivity of p38 $\delta$  inhibitors is important and require special attention, as in some types of cancer the isoforms of p38 (among themselves) may have contrasting roles.

Although studies have demonstrated that targeting DFG-out binding mode may have some advantages in p38 inhibition, it has also been shown that p38 inhibitors can bind equally well to both DFG-in and DFG-out mode, with differences in selectivity and potency. Specifically, for p38 $\delta$ , it looks like inhibitors with a DFG-out binding mode are more potent

than those with a DFG-in binding mode. This evidence leads to the conclusion that type-II inhibitors, or maybe type-1 $\frac{1}{2}$ , may be favorable in inhibiting p38 $\delta$  since they could bind in a DFG-out state. Moreover, the structure of compounds with the proposal to inhibit p38 $\delta$  needs to consider that this isoform has a bulky methionine as gatekeeper residue, which might impair the binding and selectivity of the inhibitor. Despite the limited success to obtain inhibitors specific to p38 $\delta$ , this p38 isoform remains a potential candidate for the development of new targeted therapies for cancer. The efforts to design a potent and selective inhibitor of p38 $\delta$  are important, since the lack of these compounds limits knowledge on p38 $\delta$  biological functions, as well as delaying the development of new targeted therapies based on p38 $\delta$  inhibition.

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## References

1. Plotnikov, A.; Zehorai, E.; Procaccia, S.; Seger, R. The MAPK cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. *Biochim. Biophys. Acta Mol. Cell Res.* **2011**, *1813*, 1619–1633. [[CrossRef](#)]
2. Kim, E.K.; Choi, E.J. Compromised MAPK signaling in human diseases: An update. *Arch. Toxicol.* **2015**, *89*, 867–882. [[CrossRef](#)]
3. Kim, E.K.; Choi, E.J. Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta Mol. Basis Dis.* **2010**, *1802*, 396–405. [[CrossRef](#)]
4. Cuenda, A.; Rousseau, S. p38 MAP-Kinases pathway regulation, function and role in human diseases. *Biochim. Biophys. Acta Mol. Cell Res.* **2007**, *1773*, 1358–1375. [[CrossRef](#)] [[PubMed](#)]
5. Cuadrado, A.; Nebreda, A.R. Mechanisms and functions of p38 MAPK signalling. *Biochem. J.* **2010**, *429*, 403–417. [[CrossRef](#)] [[PubMed](#)]
6. Remy, G.; Risco, A.M.; Iñesta-Vaquera, F.A.; González-Terán, B.; Sabio, G.; Davis, R.J.; Cuenda, A. Differential activation of p38MAPK isoforms by MKK6 and MKK3. *Cell. Signal.* **2010**, *22*, 660–667. [[CrossRef](#)] [[PubMed](#)]
7. Brancho, D.; Tanaka, N.; Jaeschke, A.; Ventura, J.J.; Kelkar, N.; Tanaka, Y.; Kyuuma, M.; Takeshita, T.; Flavell, R.A.; Davis, R.J. Mechanism of p38 MAP kinase activation in vivo. *Genes Dev.* **2003**, *17*, 1969–1978. [[CrossRef](#)] [[PubMed](#)]
8. Yurtsever, Z.; Scheaffer, S.M.; Romero, A.G.; Holtzman, M.J.; Brett, T.J. The crystal structure of phosphorylated MAPK13 reveals common structural features and differences in p38 MAPK family activation. *Acta Cryst. Sect. D Biol. Crystallogr.* **2015**, *71*, 790–799. [[CrossRef](#)] [[PubMed](#)]
9. Del Barco Barrantes, I.; Coya, J.M.; Maina, F.; Arthurd, J.S.C.; Nebreda, A.R. Genetic analysis of specific and redundant roles for p38 $\alpha$  and p38 $\beta$  MAPKs during mouse development. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 12764–12769. [[CrossRef](#)]
10. Goedert, M.; Cuenda, A.; Craxton, M.; Jakes, R.; Cohen, P. Activation of the novel stress-activated protein kinase SAPK4 by cytokines and cellular stresses is mediated by SKK3 (MKK6); comparison of its substrate specificity with that of other SAP kinases. *EMBO J.* **1997**, *16*, 3563–3571. [[CrossRef](#)]
11. Goedert, M.; Hasegawa, M.; Jakes, R.; Lawler, S.; Cuenda, A.; Cohen, P. Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. *FEBS Lett.* **1997**, *409*, 57–62. [[CrossRef](#)]
12. Koul, H.K.; Pal, M.; Koul, S. Role of p38 MAP Kinase Signal Transduction in Solid Tumors. *Genes Cancer* **2013**, *4*, 342–359. [[CrossRef](#)]
13. Martínez-Limón, A.; Joaquin, M.; Caballero, M.; Posas, F.; de Nadal, E. The p38 pathway: From biology to cancer therapy. *Int. J. Mol. Sci.* **2020**, *21*, 1913. [[CrossRef](#)] [[PubMed](#)]
14. Adams, R.H.; Porras, A.; Alonso, G.; Jones, M.; Vintersten, K.; Panelli, S.; Valladares, A.; Perez, L.; Klein, R.; Nebreda, A.R. Essential role of p38 $\alpha$  MAP kinase in placental but not embryonic cardiovascular development. *Mol. Cell* **2000**, *6*, 109–116. [[CrossRef](#)]
15. Mudgett, J.S.; Ding, J.; Guh-Siesel, L.; Chartrain, N.A.; Yang, L.; Gopal, S.; Shen, M.M. Essential role for p38 $\alpha$  mitogen-activated protein kinase in placental angiogenesis. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 10454–10459. [[CrossRef](#)] [[PubMed](#)]

16. Sabio, G.; Arthur, J.S.C.; Kuma, Y.; Peggie, M.; Carr, J.; Murray-Tait, V.; Centeno, F.; Goedert, M.; Morrice, N.A.; Cuenda, A. p38 $\gamma$  regulates the localisation of SAP97 in the cytoskeleton by modulating its interaction with GKAP. *EMBO J.* **2005**, *24*, 1134–1145. [[CrossRef](#)]
17. Sumara, G.; Formentini, I.; Collins, S.; Sumara, I.; Windak, R.; Bodenmiller, B.; Ramracheya, R.; Caille, D.; Jiang, H.; Platt, K.A.; et al. Regulation of PKD by the MAPK p38 $\delta$  in Insulin Secretion and Glucose Homeostasis. *Cell* **2009**, *136*, 235–248. [[CrossRef](#)] [[PubMed](#)]
18. Risco, A.; del Fresno, C.; Mambol, A.; Alsina-Beauchamp, D.; MacKenzie, K.F.; Yang, H.-T.; Barber, D.F.; Morcelle, C.; Arthur, J.S.C.; Ley, S.C.; et al. p38 $\gamma$  and p38 $\delta$  kinases regulate the Toll-like receptor 4 (TLR4)-induced cytokine production by controlling ERK1/2 protein kinase pathway activation. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11200–11205. [[CrossRef](#)]
19. Alevy, Y.G.; Patel, A.C.; Romero, A.G.; Patel, D.A.; Tucker, J.; Roswit, W.T.; Miller, C.A.; Heier, R.F.; Byers, D.E.; Brett, T.J.; et al. IL-13-induced airway mucus production is attenuated by MAPK13 inhibition. *J. Clin. Investig.* **2012**, *122*, 4555–4568. [[CrossRef](#)]
20. Cuenda, A.; Sanz-Ezquerro, J.J. p38 $\gamma$  and p38 $\delta$ : From Spectators to Key Physiological Players. *Trends Biochem. Sci.* **2017**, *42*, 431–442. [[CrossRef](#)]
21. Wang, X.S.; Diener, K.; Manthey, C.L.; Wang, S.-W.; Rosenzweig, B.; Bray, J.; Delaney, J.; Cole, C.N.; Chan-Hui, P.-Y.; Mantlo, N.; et al. Molecular Cloning and Characterization of a Novel p38 Mitogen-activated Protein Kinase. *J. Biol. Chem.* **1997**, *272*, 23668–23674. [[CrossRef](#)]
22. Escós, A.; Risco, A.; Alsina-Beauchamp, D.; Cuenda, A. p38 $\gamma$  and p38 $\delta$  Mitogen Activated Protein Kinases (MAPKs), New Stars in the MAPK Galaxy. *Front. Cell Dev. Biol.* **2016**, *4*, 31. [[CrossRef](#)] [[PubMed](#)]
23. Feijoo, C.; Campbell, D.G.; Jakes, R.; Goedert, M.; Cuenda, A. Evidence that phosphorylation of the microtubule-associated protein Tau by SAPK4/p38 $\delta$  at Thr50 promotes microtubule assembly. *J. Cell Sci.* **2005**, *118*, 397–408. [[CrossRef](#)] [[PubMed](#)]
24. Johansen, C.; Kragballe, K.; Westergaard, M.; Henningsen, J.; Kristiansen, K.; Iversen, L. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br. J. Dermatol.* **2005**, *152*, 37–42. [[CrossRef](#)]
25. Criado, G.; Risco, A.; Alsina-Beauchamp, D.; Pérez-Lorenzo, M.J.; Escós, A.; Cuenda, A. Alternative p38 MAPKs are essential for collagen-induced arthritis. *Arthritis Rheumatol.* **2014**, *66*, 1208–1217. [[CrossRef](#)]
26. Tan, F.L.S.; Ooi, A.; Huang, D.; Wong, J.C.; Qian, C.N.; Chao, C.; Ooi, L.; Tan, Y.M.; Chung, A.; Cheow, P.C.; et al. p38delta/MAPK13 as a diagnostic marker for cholangiocarcinoma and its involvement in cell motility and invasion. *Int. J. Cancer* **2010**, *126*, 2353–2361. [[CrossRef](#)]
27. Wada, M.; Canals, D.; Adada, M.; Arthur, J.S.; Hannun, Y.A.; Salama, M.F.; Coant, N.; Obeid, L.M.; Shroyer, K.R.; Kitatani, K.; et al. P38 delta MAPK promotes breast cancer progression and lung metastasis by enhancing cell proliferation and cell detachment. *Oncogene* **2017**, *36*, 6649–6657. [[CrossRef](#)] [[PubMed](#)]
28. Sahu, V.; Nigam, L.; Agnihotri, V.; Gupta, A.; Shekhar, S.; Subbarao, N.; Bhaskar, S.; Dey, S. Diagnostic Significance of p38 Isoforms (p38 $\alpha$ , p38 $\beta$ , p38 $\gamma$ , p38 $\delta$ ) in Head and Neck Squamous Cell Carcinoma: Comparative serum level evaluation and design of novel peptide inhibitor targeting the same. *Cancer Res. Treat.* **2019**, *51*, 313–325. [[CrossRef](#)] [[PubMed](#)]
29. Kiss, A.; Koppel, A.C.; Murphy, E.; Sall, M.; Barlas, M.; Kissling, G.; Efimova, T. Cell type-specific p38 $\delta$  targeting reveals a context-, stage-, and sex-dependent regulation of skin carcinogenesis. *Int. J. Mol. Sci.* **2019**, *20*, 1532. [[CrossRef](#)] [[PubMed](#)]
30. Junttila, M.R.; Ala-Aho, R.; Jokilehto, T.; Peltonen, J.; Kallajoki, M.; Grenman, R.; Jaakkola, P.; Westermarck, J.; Kähäri, V.M. p38 $\alpha$  and p38 $\delta$  mitogen-activated protein kinase isoforms regulate invasion and growth of head and neck squamous carcinoma cells. *Oncogene* **2007**, *26*, 5267–5279. [[CrossRef](#)] [[PubMed](#)]
31. Cerezo-guisado, M.I.; del Reino, P.; Remy, G.; Kuma, Y.; Arthur, J.S.C.; Gallego-Ortega, D.; Cuenda, A. Evidence of p38 $\gamma$  and p38 $\delta$  involvement in cell transformation processes. *Carcinogenesis* **2011**, *32*, 1093–1099. [[CrossRef](#)]
32. O’Callaghan, C.; Fanning, L.J.; Houston, A.; Barry, O.P. Loss of p38 $\delta$  expression promotes oesophageal squamous cell carcinoma proliferation, migration and anchorage-independent growth. *Int. J. Oncol.* **2013**, *43*, 405–415. [[CrossRef](#)]
33. Del Reino, P.; Alsina-Beauchamp, D.; Escós, A.; Cerezo-Guisado, M.I.; Risco, A.; Aparicio, N.; Zur, R.; Fernandez-Estévez, M.; Collantes, E.; Montans, J.; et al. Pro-oncogenic role of alternative p38 mitogen-activated protein kinases p38 $\gamma$  and p38 $\delta$ , linking inflammation and cancer in colitis-associated colon cancer. *Cancer Res.* **2014**, *74*, 6150–6160. [[CrossRef](#)]
34. Wu, P.; Nielsen, T.E.; Clausen, M.H. FDA-approved small-molecule kinase inhibitors. *Trends Pharmacol. Sci.* **2015**, *36*, 422–439. [[CrossRef](#)]
35. Haller, V.; Nahidino, P.; Forster, M.; Laufer, S.A. An updated patent review of p38 MAP kinase inhibitors (2014–2019). *Expert Opin. Ther. Pat.* **2020**, *30*, 453–466. [[CrossRef](#)] [[PubMed](#)]
36. Alonso, G.; Ambrosino, C.; Jones, M.; Nebreda, A.R. Differential activation of p38 mitogen-activated protein kinase isoforms depending on signal strength. *J. Biol. Chem.* **2000**, *275*, 40641–40648. [[CrossRef](#)]
37. Risco, A.; Cuenda, A. New Insights into the p38  $\gamma$  and p38  $\delta$  MAPK Pathways. *J. Signal Transduct.* **2012**, *2012*, 1–8. [[CrossRef](#)] [[PubMed](#)]
38. Liu, Q.; Zou, R.; Zhou, R.; Gong, C.; Wang, Z.; Cai, T.; Tan, C.; Fang, J. MiR-155 regulates glioma cells invasion and chemosensitivity by p38 isoforms in vitro. *J. Cell. Biochem.* **2015**, *116*, 1213–1221. [[CrossRef](#)]
39. Winer, A.; Adams, S.; Mignatti, P. Matrix metalloproteinase inhibitors in cancer therapy: Turning past failures into future successes. *Mol. Cancer Ther.* **2018**, *17*, 1147–1155. [[CrossRef](#)] [[PubMed](#)]

40. Webb, A.H.; Gao, B.T.; Goldsmith, Z.K.; Irvine, A.S.; Saleh, N.; Lee, R.P.; Lendermon, J.B.; Bheemreddy, R.; Zhang, Q.; Brennan, R.C.; et al. Inhibition of MMP-2 and MMP-9 decreases cellular migration, and angiogenesis in in vitro models of retinoblastoma. *BMC Cancer* **2017**, *17*. [[CrossRef](#)]
41. O'Callaghan, C.; Fanning, L.J.; Barry, O.P. p38 $\delta$  MAPK phenotype: An indicator of chemotherapeutic response in oesophageal squamous cell carcinoma. *Anticancer Drugs* **2015**, *26*, 46–55. [[CrossRef](#)]
42. O'Callaghan, C.; Fanning, L.J.; Barry, O.P. Hypermethylation of MAPK13 promoter in oesophageal squamous cell carcinoma is associated with loss of p38 $\delta$  MAPK expression. *Cancers* **2015**, *7*, 2124–2133. [[CrossRef](#)]
43. Gao, L.; Smit, M.A.; van den Oord, J.J.; Goeman, J.J.; Verdegaal, E.M.E.; van der Burg, S.H.; Stas, M.; Beck, S.; Gruis, N.A.; Tensen, C.P.; et al. Genome-wide promoter methylation analysis identifies epigenetic silencing of MAPK13 in primary cutaneous melanoma. *Pigment Cell Melanoma Res.* **2013**, *26*, 542–554. [[CrossRef](#)]
44. Schindler, E.M.; Hindes, A.; Gribben, E.L.; Burns, C.J.; Yin, Y.; Lin, M.H.; Owen, R.J.; Longmore, G.D.; Kissling, G.E.; Arthur, J.S.C.; et al. p38 $\delta$  mitogen-activated protein kinase is essential for skin tumor development in mice. *Cancer Res.* **2009**, *69*, 4648–4655. [[CrossRef](#)]
45. Zur, R.; Garcia-Ibanez, L.; Nunez-Buiza, A.; Aparicio, N.; Liappas, G.; Escós, A.; Risco, A.; Page, A.; Saiz-Ladera, C.; Alsina-Beauchamp, D.; et al. Combined deletion of p38 $\gamma$  and p38 $\delta$  reduces skin inflammation and protects from carcinogenesis. *Oncotarget* **2015**, *6*, 12920–12935. [[CrossRef](#)] [[PubMed](#)]
46. Zhong, J.; Lardinois, D.; Szilard, J.; Tamm, M.; Roth, M. Rat mesothelioma cell proliferation requires p38 $\delta$  mitogen activated protein kinase and C/EBP- $\alpha$ . *Lung Cancer* **2011**, *73*, 166–170. [[CrossRef](#)] [[PubMed](#)]
47. Yu, J.X.; Craig, A.J.; Duffy, M.E.; Villacorta-Martin, C.; Miguela, V.; de Galarreta, M.R.; Scopton, A.P.; Silber, L.; Maldonado, A.Y.; Rialdi, A.; et al. Phenotype-based screens with conformation-specific inhibitors reveal p38 gamma and delta as targets for HCC polypharmacology. *Mol. Cancer Ther.* **2019**, *18*, 1506–1519. [[CrossRef](#)] [[PubMed](#)]
48. Kim, D.J.; Chan, K.S.; Sano, S.; DiGiovanni, J. Signal transducer and activator of transcription 3 (Stat3) in epithelial carcinogenesis. *Mol. Carcinog.* **2007**, *46*, 725–731. [[CrossRef](#)]
49. Park, C.Y.; Tseng, D.; Weissman, I.L. Cancer stem cell-directed therapies: Recent data from the laboratory and clinic. *Mol. Ther.* **2009**, *17*, 219–230. [[CrossRef](#)] [[PubMed](#)]
50. Barbato, L.; Bocchetti, M.; Di Biase, A.; Regad, T. Cancer Stem Cells and Targeting Strategies. *Cells* **2019**, *8*, 926. [[CrossRef](#)] [[PubMed](#)]
51. Yasuda, K.; Hirohashi, Y.; Kuroda, T.; Takaya, A.; Kubo, T.; Kanaseki, T.; Tsukahara, T.; Hasegawa, T.; Saito, T.; Sato, N.; et al. MAPK13 is preferentially expressed in gynecological cancer stem cells and has a role in the tumor-initiation. *Biochem. Biophys. Res. Commun.* **2016**, *472*, 643–647. [[CrossRef](#)]
52. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [[CrossRef](#)] [[PubMed](#)]
53. Grivnickov, S.I.; Greten, F.R.; Karin, M. Immunity, Inflammation, and Cancer. *Cell* **2010**, *140*, 883–899. [[CrossRef](#)]
54. Ittner, A.; Block, H.; Reichel, C.A.; Varjosalo, M.; Gehart, H.; Sumara, G.; Gstaiger, M.; Krombach, F.; Zarbock, A.; Ricci, R. Regulation of PTEN activity by p38 $\delta$ -PKD1 signaling in neutrophils confers inflammatory responses in the lung. *J. Exp. Med.* **2012**, *209*, 2229–2246. [[CrossRef](#)]
55. Barrio, L.; Román-García, S.; Díaz-Mora, E.; Risco, A.; Jiménez-Saiz, R.; Carrasco, Y.R.; Cuenda, A. B Cell Development and T-Dependent Antibody Response Are Regulated by p38 $\gamma$  and p38 $\delta$ . *Front. Cell Dev. Biol.* **2020**, *8*, 1–15. [[CrossRef](#)] [[PubMed](#)]
56. Kitatani, K.; Sheldon, K.; Anelli, V.; Jenkins, R.W.; Sun, Y.; Grabowski, G.A.; Obeid, L.M.; Hannun, Y.A. Acid  $\beta$ -Glucosidase 1 Counteracts p38 $\delta$ -dependent Induction of Interleukin-6: Possible role for ceramide as an anti-inflammatory lipid. *J. Biol. Chem.* **2009**, *284*, 12979–12988. [[CrossRef](#)] [[PubMed](#)]
57. Wu, Y.; Zhou, B.P. Inflammation: A driving force speeds cancer metastasis. *Cell Cycle* **2009**, *8*, 3267–3273. [[CrossRef](#)] [[PubMed](#)]
58. Crusz, S.M.; Balkwill, F.R. Inflammation and cancer: Advances and new agents. *Nat. Rev. Clin. Oncol.* **2015**, *12*, 584–596. [[CrossRef](#)] [[PubMed](#)]
59. Holen, I.; Lefley, D.V.; Francis, S.E.; Rennicks, S.; Bradbury, S.; Coleman, R.E.; Ottewell, P. IL-1 drives breast cancer growth and bone metastasis in vivo. *Oncotarget* **2016**, *7*, 75571–75584. [[CrossRef](#)] [[PubMed](#)]
60. Hanahan, D.; Weinberg, R.A. The Hallmarks of Cancer. *Cell* **2000**, *100*, 57–70. [[CrossRef](#)]
61. Yamada, T.; Oshima, T.; Yoshihara, K.; Tamura, S.; Kanazawa, A.; Inagaki, D.; Yamamoto, N.; Sato, T.; Fujii, S.; Numata, K.; et al. Overexpression of MMP-13 gene in colorectal cancer with liver metastasis. *Anticancer Res.* **2010**, *30*, 2693–2699.
62. Wang, J.; Ye, C.; Lu, D.; Chen, Y.; Jia, Y.; Ying, X.; Xiong, H.; Zhao, W.; Zhou, J.; Wang, L. Matrix metalloproteinase-1 expression in breast carcinoma: A marker for unfavorable prognosis. *Oncotarget* **2017**, *8*, 91379–91390. [[CrossRef](#)] [[PubMed](#)]
63. Tian, R.; Li, X.; Gao, Y.; Li, Y.; Yang, P.; Wang, K. Identification and validation of the role of matrix metalloproteinase-1 in cervical cancer. *Int. J. Oncol.* **2018**, *52*, 1198–1208. [[CrossRef](#)]
64. Gonzalez-Avila, G.; Sommer, B.; Mendoza-Posada, D.A.; Ramos, C.; Garcia-Hernandez, A.A.; Falfan-Valencia, R. Matrix metalloproteinases participation in the metastatic process and their diagnostic and therapeutic applications in cancer. *Crit. Rev. Oncol. Hematol.* **2019**, *137*, 57–83. [[CrossRef](#)] [[PubMed](#)]
65. Shi, D.B.; Ma, R.R.; Zhang, H.; Hou, F.; Guo, X.Y.; Gao, P. GAGE7B promotes tumor metastasis and growth via activating the p38 $\delta$ /pMAPKAPK2/pHSP27 pathway in gastric cancer. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 124. [[CrossRef](#)] [[PubMed](#)]
66. Kato, K.; Ito, H.; Iwamoto, I.; Iida, K.; Inaguma, Y. Protein kinase inhibitors can suppress stress-induced dissociation of Hsp27. *Cell Stress Chaperones* **2001**, *6*, 16–20. [[CrossRef](#)]

67. Nakashima, M.; Adachi, S.; Yasuda, I.; Yamauchi, T.; Kawaguchi, J.; Itani, M.; Yoshioka, T.; Matsushima-Nishiwaki, R.; Hirose, Y.; Kozawa, O.; et al. Phosphorylation status of heat shock protein 27 plays a key role in gemcitabine-induced apoptosis of pancreatic cancer cells. *Cancer Lett.* **2011**, *313*, 218–225. [[CrossRef](#)]
68. Lambert, A.W.; Pattabiraman, D.R.; Weinberg, R.A. Emerging Biological Principles of Metastasis. *Cell* **2017**, *168*, 670–691. [[CrossRef](#)]
69. Lee, M.; Dominguez, C. MAP Kinase p38 Inhibitors: Clinical Results and an Intimate Look at Their Interactions with p38 $\alpha$  Protein. *Curr. Med. Chem.* **2005**, *12*, 2979–2994. [[CrossRef](#)]
70. Murphy, A.G.; Zheng, L. Small molecule drugs with immunomodulatory effects in cancer. *Hum. Vaccines Immunother.* **2015**, *11*, 2463–2468. [[CrossRef](#)]
71. Roskoski, R. Properties of FDA-approved small molecule protein kinase inhibitors: A 2020 update. *Pharmacol. Res.* **2020**, *152*, 104609. [[CrossRef](#)] [[PubMed](#)]
72. Zhang, J.; Shen, B.; Lin, A. Novel strategies for inhibition of the p38 MAPK pathway. *Trends Pharmacol. Sci.* **2007**, *28*, 286–295. [[CrossRef](#)]
73. Tong, M.; Seeliger, M.A. Targeting conformational plasticity of protein kinases. *ACS Chem. Biol.* **2015**, *10*, 190–200. [[CrossRef](#)] [[PubMed](#)]
74. Kumar, G.S.; Clarkson, M.W.; Kunze, M.B.A.; Granata, D.; Joshua Wand, A.; Lindorff-Larsen, K.; Page, R.; Peti, W. Dynamic activation and regulation of the mitogen-activated protein kinase p38. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4655–4660. [[CrossRef](#)] [[PubMed](#)]
75. Huse, M.; Kuriyan, J. The Conformational Plasticity of Protein Kinases. *Cell* **2002**, *109*, 275–282. [[CrossRef](#)]
76. Filomia, F.; De Rienzo, F.; Menziani, M.C. Insights into MAPK p38 $\alpha$  DFG flip mechanism by accelerated molecular dynamics. *Bioorganic Med. Chem.* **2010**, *18*, 6805–6812. [[CrossRef](#)]
77. Ung, P.M.U.; Schlessinger, A. DFGmodel: Predicting protein kinase structures in inactive states for structure-based discovery of type-II inhibitors. *ACS Chem. Biol.* **2015**, *10*, 269–278. [[CrossRef](#)]
78. Zuccotto, F.; Ardini, E.; Casale, E.; Angiolini, M. Through the “gatekeeper door”: Exploiting the active kinase conformation. *J. Med. Chem.* **2010**, *53*, 2681–2694. [[CrossRef](#)]
79. Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P.F.; Gilmore, T.; Graham, A.G.; Grob, P.M.; Hickey, E.R.; Moss, N.; Pav, S.; et al. Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat. Struct. Biol.* **2002**, *9*, 268–272. [[CrossRef](#)]
80. Zhang, J.; Yang, P.L.; Gray, N.S. Targeting cancer with small molecule kinase inhibitors. *Nat. Rev. Cancer* **2009**, *9*, 28–39. [[CrossRef](#)]
81. Koeberle, S.C.; Romir, J.; Fischer, S.; Koeberle, A.; Schattel, V.; Albrecht, W.; Grütter, C.; Werz, O.; Rauh, D.; Stehle, T.; et al. Skepinone-L is a selective p38 mitogen-activated protein kinase inhibitor. *Nat. Chem. Biol.* **2012**, *8*, 141–143. [[CrossRef](#)] [[PubMed](#)]
82. Wang, Z.; Canagarajah, B.J.; Boehm, J.C.; Kassisà, S.; Cobb, M.H.; Young, P.R.; Abdel-Meguid, S.; Adams, J.L.; Goldsmith, E.J. Structural basis of inhibitor selectivity in MAP kinases. *Structure* **1998**, *6*, 1117–1128. [[CrossRef](#)]
83. Kufareva, I.; Abagyan, R. Type-II kinase inhibitor docking, screening, and profiling using modified structures of active kinase states. *J. Med. Chem.* **2009**, *51*, 7921–7932. [[CrossRef](#)] [[PubMed](#)]
84. Wentsch, H.K.; Walter, N.M.; Bührmann, M.; Mayer-Wrangowski, S.; Rauh, D.; Zaman, G.J.R.; Willemsen-Seegers, N.; Buijsman, R.C.; Henning, M.; Dauch, D.; et al. Optimized Target Residence Time: Type II/2 Inhibitors for p38 $\alpha$  MAP Kinase with Improved Binding Kinetics through Direct Interaction with the R-Spine. *Angew. Chem. Int. Ed.* **2017**, *56*, 5363–5367. [[CrossRef](#)] [[PubMed](#)]
85. Walter, N.M.; Wentsch, H.K.; Bührmann, M.; Bauer, S.M.; Döring, E.; Mayer-Wrangowski, S.; Sievers-Engler, A.; Willemsen-Seegers, N.; Zaman, G.; Buijsman, R.; et al. Design, Synthesis, and Biological Evaluation of Novel Type II/2 p38 $\alpha$  MAP Kinase Inhibitors with Excellent Selectivity, High Potency, and Prolonged Target Residence Time by Interfering with the R-Spine. *J. Med. Chem.* **2017**, *60*, 8027–8054. [[CrossRef](#)]
86. Kondoh, Y.; Honda, K.; Hiranuma, S.; Hayashi, T.; Shimizu, T.; Watanabe, N.; Osada, H. Comparative chemical array screening for p38 $\pi$ / $\sigma$  MAPK inhibitors using a single gatekeeper residue difference between p38 $\alpha$ / $\beta$  and p38 $\pi$ / $\sigma$ . *Sci. Rep.* **2016**, *6*, 29881. [[CrossRef](#)]
87. Eysers, P.A.; Craxton, M.; Morrice, N.; Cohen, P.; Goedert, M. Conversion of SB 203580-insensitive MAP kinase family members to drug-sensitive forms by a single amino-acid substitution. *Chem. Biol.* **1998**, *5*, 321–328. [[CrossRef](#)]
88. Yurtsever, Z.; Patel, D.A.; Kober, D.L.; Su, A.; Miller, C.A.; Romero, A.G.; Holtzman, M.J.; Brett, T.J. First comprehensive structural and biophysical analysis of MAPK13 inhibitors targeting DFG-in and DFG-out binding modes. *Biochim. Biophys. Acta Gen. Subj.* **2016**, *1860*, 2335–2344. [[CrossRef](#)]
89. Stramucci, L.; Bossi, G. Approaching the challenges of MKK3/p38 $\delta$  MAPK targeting for therapeutic purpose in colorectal cancer. *J. Exp. Clin. Cancer Res.* **2019**, *38*, 19–21. [[CrossRef](#)]
90. Stramucci, L.; Pranteda, A.; Stravato, A.; Amoreo, C.A.; Pennetti, A.; Diodoro, M.G.; Bartolazzi, A.; Milella, M.; Bossi, G. MKK3 sustains cell proliferation and survival through p38 $\Delta$  MAPK activation in colorectal cancer. *Cell Death Dis.* **2019**, *10*, 842. [[CrossRef](#)]