Signal integration by JNK and p38 MAPK pathways in cancer development

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Abstract | Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) family members function in a cell context-specific and cell type-specific manner to integrate signals that affect proliferation, differentiation, survival and migration. Consistent with the importance of these events in tumorigenesis, JNK and p38 MAPK signalling is associated with cancers in humans and mice. Studies in mouse models have been essential to better understand how these MAPKs control cancer development, and these models are expected to provide new strategies for the design of improved therapeutic approaches. In this Review we highlight the recent progress made in defining the functions of the JNK and p38 MAPK pathways in different cancers.

Mitogen-activated protein kinases (MAPKs) are signalling components that are important in converting extracellular stimuli into a wide range of cellular responses. The ERK1 and ERK2 MAPKs are activated by mitogens and were found to be upregulated in human tumours; this finding has led to the development of inhibitors of this pathway for cancer therapeutics[1]. Two other major MAPK pathways, the Jun N-terminal kinase (JNK) and p38 MAPK pathways, which are also called stress-activated protein kinase pathways, are also often deregulated in cancers. JNKs and p38 MAPKs are activated by environmental and genotoxic stresses and have key roles in inflammation, as well as in tissue homeostasis, as they control cell proliferation, differentiation, survival and the migration of specific cell types[2–6]. The functions of JNKs and p38 MAPKs in cancer development are complex, which is consistent with the wide range of cellular responses that they modulate. Certain cells use these signalling pathways to antagonize cell proliferation and morphological transformation, whereas cancer cells can subvert these pathways to facilitate proliferation, survival and invasion. A molecular understanding of how JNK and p38 MAPK family members and their isoforms function either as tumour suppressors or oncoproteins in specific cell types is largely missing. Moreover, the extent of redundancy and crosstalk between these signalling pathways and the consequent physiological implications are poorly understood. One major challenge will be to determine how, when and where specific targeting of the JNK and p38 MAPK pathways should be considered for therapeutic applications. This Review aims to describe the progress made in determining the role of JNK and p38 MAPK signalling in cancers, drawing together insights from mouse models and human cancer, and also attempts to answer some of the open questions remaining in the field of MAPK signalling.

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doi:10.1038/nrc2694

Signalling by MAPKs

MAPKs are evolutionarily conserved enzymes, the activation of which requires dual phosphorylation on the Thr-X-Tyr motif that is catalysed by MAP2K kinases (FIG. 1). After activation, MAPKs phosphorylate specific serine and threonine residues of target substrates, which include other protein kinases and many transcription factors. MAPKs are switched off by both generic phosphatases and dual-specificity phosphatases and are further regulated by scaffold proteins, which are usually specific for each of the three major mammalian MAPK pathways[7–9].

JNK signalling. The JNK proteins are encoded by three genes, *MAPK8* (which encodes JNK1), *MAPK9* (which encodes JNK2) and *MAPK10* (which encodes JNK3), which are alternatively spliced giving rise to at least ten isoforms[10]. JNK1 and JNK2 are expressed in almost every cell, whereas JNK3 is mainly found in the brain[11,12]. JNKs can be activated by the upstream MKK4 and MKK7 kinases (FIG. 1). Although there are many JNK substrates, it is still a challenge to identify the molecular networks regulated by the individual JNK family members[4–6].

At a glance

- Jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinases (MAPKs) have important roles in the signalling mechanisms that orchestrate cellular responses to many types of stresses, but also control the proliferation, differentiation, survival and migration of specific cell types.
- JNKs and p38 MAPKs can exert antagonistic effects on cell proliferation and survival, which depend on cell type-specific differences, as well as on the intensity and duration of the signal and the crosstalk between other signalling pathways.
- Crosstalk between the JNK and p38 MAPK pathways is emerging as an important regulatory mechanism in many cellular responses.
- The JNK and p38 MAPK pathways regulate the activity and expression of key inflammatory mediators, including cytokines and proteases, which may function as potent cancer promoters.
- The specific role of individual JNK and p38 MAPK family members in particular cellular processes *in vivo* has been addressed by gene-targeting experiments in mice. Genetically engineered mouse models have confirmed the importance of these pathways for tumorigenesis in various organs.
- The expression or activity of JNK and p38 MAPK pathway components is often altered in human tumours and cancer cell lines. Given the many tumorigenesis-related functions that these kinases can control, both in the cancer cell and in the tumour microenvironment, it is important to carefully consider the type of tumour before attempting to modulate these pathways for cancer therapy.

A major JNK target is the transcription factor AP1, which is composed of Fos and Jun family members[13]. The oncogenic functions of JNKs are mostly based on their ability to phosphorylate JUN and to activate AP1, whereas their tumour-suppressive functions are probably related to their pro-apoptotic activity. The JNK/JUN pathway regulates a plethora of target genes that contain AP1-binding sites, including genes that control the cell cycle, as well as survival and apoptosis, metalloproteinases and nuclear hormone receptors, such as retinoid receptors[14]. In addition, factors such as the signal intensity and crosstalk between different JNK isoforms are important for the overall effect of JNK activation on tumour development[4,5,12].

p38 MAPK signalling. There are four genes that encode p38 MAPKs: *MAPK14* (which encodes p38α), *MAPK11* (which encodes p38β), *MAPK12* (which encodes p38γ) and *MAPK13* (which encodes p38δ); two alternatively spliced isoforms of *MAPK14* have also been reported[15,16]. p38α and p38β are closely related proteins that could have overlapping functions. Whereas p38α is highly abundant in most cell types, p38β seems to be expressed at very low levels, and its contribution to p38 MAPK signalling is not clear. p38γ and p38δ have more restricted expression patterns and are likely to have specialized functions. Most of the published literature on p38 MAPKs refers to p38α[17,18].

p38 MAPKs are activated by the upstream MKK3 and MKK6 kinases, and sometimes by MKK4 (FIG. 1); autophosphorylation may also contribute to p38 MAPK activation[18,19]. The two major groups of proteins that are regulated by p38 MAPK-mediated phosphorylation are transcription factors, such as p53, activating transcription factor 2 (ATF2), ELK1, myocyte-specific enhancer factor 2 (MEF2) and C/EBPβ; and protein kinases, including MAPK-activated kinase 2 (MK2; also known as MAPK2), mitogen- and stress-activated protein kinase 1 (MSK1), MAP kinase-interacting serine/threonine kinase 1 (MNK1) and MNK2 (REFS 17,18) (FIG. 1). There is much evidence to support a role for p38α as a tumour suppressor, and this function of p38α is mostly mediated by both negative regulation of cell cycle progression and the induction of apoptosis, although the induction of terminal differentiation also contributes to its tumour-suppressive function[20–22]. However, p38α may also have oncogenic functions that are mediated by its involvement in key processes of cancer progression, such as invasion, inflammation and angiogenesis.

Proliferation, survival and differentiation

Unscheduled proliferation is a hallmark of cancer, and the JNK and p38 MAPK pathways regulate cell cycle progression at different transition points by both transcription-dependent and transcription-independent mechanisms. In addition, both pathways modulate the cellular programmes for cell survival and differentiation, with profound effects on the development of various cancers.

Role of JNKs. Depending on the stimuli and the strength and duration of JNK activation, the cellular response has diverse outcomes, which range from the induction of apoptosis to increased survival and altered proliferation. As studies on JNK activation use a wide range of experimental settings and genetically altered cells, the messages emerging from these studies and the consequent implications for cancer development are complex[4,5,12,22].

Because different JNKs differ substantially in their ability to interact with JUN, a well-established regulator of cell cycle progression[13], these kinases have profound effects on cell proliferation[5,23,24]. In non-stimulated cells, JNK2 seems to mainly target JUN for degradation, whereas following stimulation, JNK1 phosphorylates and stabilizes JUN leading to transcriptional activation[25]. Consequently, compared with wild-type fibroblasts, *JNK2*-knockout fibroblasts grow slightly faster, whereas *JNK1*-knockout cells grow slower, a phenotype that is correlated with reduced AP1 activity and decreased JUN phosphorylation[23]. The opposing roles of JNK1 and JNK2 in proliferation have also been observed in erythrocytes and keratinocytes[23]. Interestingly, it has also been proposed that increased expression of JUN and the increased proliferation of *JNK2*-knockout fibroblasts are primarily due to compensatory increases in JNK1 function[26]. Conditional genetic experiments in different tissues and cells are necessary to better define the molecular mechanism of JNK functions. Recently, the JNK pathway was linked to p53-dependent senescence using a conditional *JNK1* allele[27]. The role of the JNK/JUN pathway as a negative regulator of the p53 tumour suppressor also supports the oncogenic role of activated JNK in tumour models.

A role for JNKs in cell survival is well established, although the proposed underlying mechanisms are controversial[5]. As cytoplasmic injection of cytochrome *c* rescued the apoptotic defects of JNK-deficient fibroblasts, JNK pro-apoptotic functions were proposed to be mediated by the mitochondrial pathway[28]. JNKs can both phosphorylate and regulate the expression of several members of the Bcl-2 protein family, such as BAX

AP1
A dimeric transcription factor complex that contains members of the Jun, Fos, Atf and Maf protein families. Expression of these 'immediate early genes' is often low or undetectable in quiescent cells, but activated in minutes following extracellular stimulation, such as the addition of a growth factor or ultraviolet irradiation and other stresses.

and Bcl2 antagonist of cell death (BAD), as well as 14-3-3 proteins (FIG. 1). Phosphorylation of 14-3-3 proteins by JNKs releases pro-apoptotic proteins, such as BAX and FOXO transcription factors, from inactive complexes, thereby facilitating JNK-mediated apoptosis[5].

The pro- or anti-apoptotic effects of JNKs, which have mostly been determined using studies in fibroblasts, seem to be dependent not only on the stimuli (for example, growth factor stimulation and ultraviolet (UV) irradiation[29]) and tissue specificity, but also on the strength of the signal. Whereas transient JNK activation was shown to promote cell survival, prolonged JNK activation mediates tumour necrosis factor-α (TNFα)-dependent apoptosis, often through distinct caspase 8 activation pathways[30]. One pathway that has been suggested involves the E3 ubiquitin ligase ITCH, which degrades the caspase 8 inhibitor CASP8 and FADD-like apoptosis regulator (CFLAR; also known as FLIP) through JNK1 phosphorylation of ITCH[31]. Another mechanism for JNK-mediated apoptosis following TNFα signalling involves caspase 8-independent cleavage of BH3-interacting domain death agonist (BID), which relieves inhibition of caspase 8 activation by TNF receptor-associated factor 2 (TRAF2)– IAP1 (also known as BIRC2)[32]. However, caspase 8 can also be activated through SMAC (also known as DIABLO), which auto-degrades IAP1 and IAP2 independently of CFLAR[33].

Compared with the well-defined molecular functions of p38 MAPKs, little is known about how JNKs control cell differentiation. In development, JNK1 and JNK2 control the migration of epithelial cells during eyelid closure, a process that does not require cell proliferation and is probably mediated by phosphorylation of paxillin and F-actin polymerization[4,34]. In adult mice, JNK1, but not JNK2, was shown to modulate osteoclast formation *in vitro* in JUN-dependent and JUN-independent ways[35]. JNKs have also been assigned functions in the regulation of T lymphocyte differentiation using knockout and dominant negative JNK transgenic mice[6,36], as well as in the nervous system[4,5]. The relevance of the JNK pathway in other tissues of adult mice and in the context of tumour formation has yet to be investigated (discussed below).

Role of p38 MAPKs. p38α can negatively regulate cell cycle progression both at the G1/S and the G2/M transitions by several mechanisms, including the downregulation of cyclins, upregulation of cyclin-dependent kinase (CDK) inhibitors and modulation of the tumour suppressor p53 (REFS 37,38) (FIG. 1). Moreover, p38γ has been reported to regulate the G2 arrest induced by γ-radiation[39]. p38 MAPKs can also trigger premature senescence in primary cells — a permanent proliferative arrest induced by oncogenes, such as *HRAS* — which has been proposed to function as an anti-tumorigenic defence mechanism by inducing p53 phosphorylation and the upregulation of p16 (REF. 40). Interestingly, negative regulation of proliferation is emerging as a highly conserved and important function of p38α in various types of primary cells, including cardiomyocytes, hepatocytes, fibroblasts, haematopoietic cells and lung cells[41–43]. This effect of p38α may be mediated by negative regulation of the JNK/JUN pathway[42] or by downregulation of epidermal growth factor receptor (EGFR)[43], depending on the cell type (FIG. 2). In contrast to p38α, p38δ has been reported to mediate TPA-induced epidermal cell proliferation in mice[44].

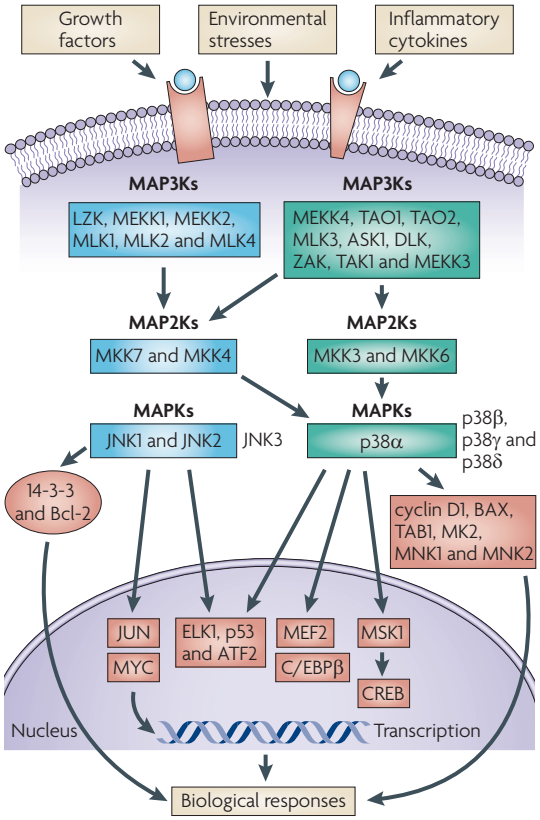

Figure 1 | **Activation of mitogen-activated protein kinase signalling pathways.** Mitogen-activated protein kinase (MAPK) pathways are activated by environmental stresses, such as ultraviolet irradiation, heat and osmotic shock, genotoxic agents, anisomycin and toxins, but are also activated following growth factor and inflammatory cytokine stimulation. The different upstream activators of Jun N-terminal kinases (JNKs) and p38 MAPKs, such as MAP2K and MAP3K family members, are depicted. In addition, downstream targets, including transcription factors and other effectors, which determine a range of biological responses from cell proliferation, survival, differentiation and migration to inflammation and cancer, are shown. Many genes are directly regulated by these transcription factors, including genes that encode p21, 14-3-3, protein phosphatase 1D (PPM1D), GADD45α and some Bcl-2 family members by p53, immediate early gene products such as FOS by ELK1, GADD45α, dual-specificity phosphatases (DUSPs), cyclin D and JUN by activating transcription factor 2 (ATF2), interleukin-6 (IL-6) and cyclooxygenase 2 (COX-2) by C/EBPβ, and DUSP1 and IL-10 by cAMP-responsive element binding protein (CREB). ASK1, apoptosis signal-regulating kinase 1; DLK, dual leucine zipper-bearing kinase; LZK, leucine-zipper kinase; MEF2, myocyte-specific enhancer factor 2; MLK, mixed-lineage kinase; MNK1, MAP kinase-interacting serine/threonine kinase 1; TAK1, transforming growth factor β-activated kinase 1; TAO, thousand-and-one amino acid kinase; ZAK, leucine-zipper and sterile-α motif kinase.

Although p38α activation is normally associated with anti-proliferative functions, there are reports — which have mainly used chemical inhibitors — indicating that p38α can sometimes positively regulate proliferation, for example in haematopoietic cells[45] and several cancer cell lines[46–51]. On a molecular level, the antagonistic effects of p38α on cell proliferation are probably attributable to different levels of kinase activity, together with the interplay between different signalling pathways[2].

The induction of apoptosis by many types of cellular stresses also involves p38α. These effects can be mediated by transcriptional and post-transcriptional mechanisms, which affect either death receptors, survival pathways or pro- and anti-apoptotic Bcl-2 proteins. The contribution of these different mechanisms to p38α-induced apoptosis is probably regulated in a stimulus- and context-dependent manner[21]. Apoptotic stimuli sometimes trigger p38α activation by secondary routes, such as the production of reactive oxygen species (ROS). This mechanism is likely to be important for the suppression of tumour initiation by p38α, which triggers apoptosis in response to the expression of ROS-inducing oncogenes in immortalized cells[52]. Notably, p38β has been proposed to have anti-apoptotic effects in various cell lines and might counteract the pro-apoptotic effect of p38α activation[53–55].

Several studies have also described pro-survival roles for p38α, which can be mediated by the induction of cell differentiation or by anti-apoptotic inflammatory signals, such as the cytokine interleukin-6 (IL-6), as well as by a quiescent state known as cancer dormancy that may be important for cancer cells to acquire drug resistance[21,56]. In addition, p38α can also mediate cell survival either by regulating autophagy programmes, through downstream targets that have not yet been characterized[57], or by direct phosphorylation and inactivation of glycogen synthase kinase 3β (GSK3β), which results in the accumulation of the transcription factor β-catenin[58]. Finally, p38α has been implicated in the G2/M checkpoint, which induces cell cycle arrest and facilitates DNA repair. This function of p38α may antagonize chemotherapy-induced DNA damage, which could also lead to apoptosis resistance in cancer cells[38].

p38α is emerging as an important regulator of differentiation programmes in many cell types, including embryonic stem cells[18,43,59,60]. It can directly phosphorylate and modulate the activity of several transcription factors involved in tissue-specific differentiation; for example, MEF2 and E47 (also known as TFE2α) in skeletal muscle or C/EBPβ in adipocytes (FIG. 1). In addition, p38α regulates the targeting of the SWI–SNF chromatin-remodelling complex to muscle promoters, which contributes to the induction of muscle-specific gene transcription[61,62]. There is evidence indicating that p38α also has a key role in the proliferation arrest that occurs at the onset of differentiation, but that it is regulated by independent mechanisms[41,43,63,64]. p38α downregulation in mice has a dramatic effect on lung homeostasis, probably reflecting the crucial function of p38α in the coordination of proliferation arrest with the induction and maintenance of the differentiation state in lung epithelial cells[42,43]. The differentiation-inducing activity of p38α may be relevant for tumour suppression, as p38α activation triggers a more differentiated and less transformed phenotype in rhabdomyosarcoma, renal carcinoma and colon cancer cell lines compared with the same cancer cell lines in which p38α has not been activated[65–67]. Importantly, the immature and poorly differentiated lung epithelium of p38α-deficient mice highly sensitizes them to KRAS-induced lung tumorigenesis[43].

In summary, the above information illustrates how the tumour suppressive mechanisms of normal cells can sometimes be switched to promote survival in cancer cells. Why p38α or JNK pathway activation induces apoptosis in some cases, but can lead to increased survival in others, is likely to depend on cell type-specific differences, together with the intensity and duration of the signal and its crosstalk with other signalling pathways.

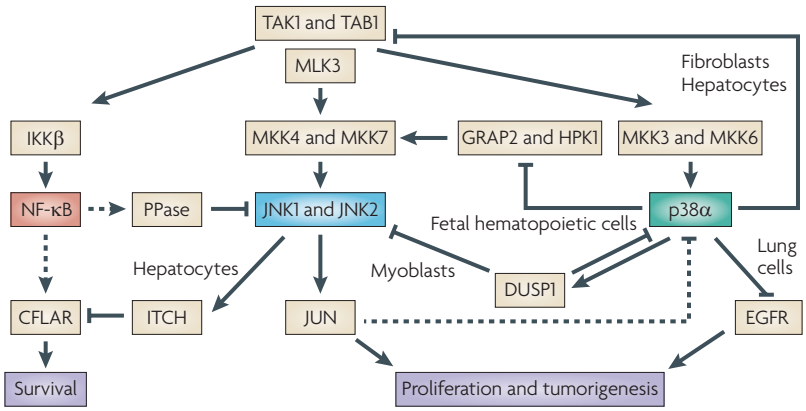

Figure 2 | **Signal integration between Jun N-terminal kinases (JNKs) and p38 mitogen-activated protein kinases (MAPKs).** The figure depicts the crosstalk between JNK, p38α and nuclear factor kappa-B (NF-κB) signalling in different cellular systems from fetal hematopoietic cells to fibroblasts, hepatocytes, myoblasts and lung cells. In each of these cellular systems different connections are established, which determine the biological response. The figure illustrates how these networks may function in a cell context- and stimulus-dependent way. Dotted lines indicate that the molecular mechanisms are not established. CFLAR, CASP8 and FADD-like apoptosis regulator; DUSP1, dual specificity protein phosphatase 1; EGFR, epidermal growth factor receptor; GRAP2, GRB2-related adaptor protein 2; IKKβ, inhibitor of nuclear factor κB kinase subunit-β; PPase; protein phosphatase.

Crosstalk between JNK and p38 MAPK pathways

Crosstalk between different signalling pathways is a common theme in cell regulation, which is usually highly dependent on cell context. The JNK and p38 MAPK pathways share several upstream regulators, and accordingly there are multiple stimuli that simultaneously activate both pathways[11] (FIG. 1). Indeed, JNKs and p38 MAPKs can potentially synergize to induce AP1 transcriptional activity, as p38α sometimes mediates the expression of both JUN and its partner FOS[68]. Nevertheless, the two stress-activated signalling pathways often have opposite effects; for example, in the regulation of cardiomyocyte hypertrophy[69], in CD95-induced Jurkat cell apoptosis[70] and in mouse models of liver cancer[42] (FIG. 2). Further evidence for the antagonism between JNK and p38 MAPKs has recently been reported in mouse

embryonic fibroblasts (MEFs) deficient in the JNK activator MKK7 (REF. 71). The impaired activation of JNKs in *MKK7*$^{-/-}$ MEFs correlates with reduced cell proliferation, augmented premature senescence and resistance to oncogenic transformation. Interestingly, all of these phenotypes were reverted by treating the MEFs with inhibitors of p38α and p38β[71]. Similar results were obtained in the context of embryonic hepatoblast proliferation *in vitro*, as well as liver regeneration after partial hepatectomy *in vivo*. The authors proposed that one of the mechanisms underlying this crosstalk might be related to the antagonistic regulation of CDK1–cyclin B kinase activity by JNKs and p38 MAPKs[71].

In addition to the crosstalk at the level of downstream targets, there is evidence indicating that the p38 MAPK pathway can negatively regulate JNK activity in several contexts (FIG. 2). The first evidence for this crosstalk was the observation that chemical inhibition of p38α and p38β strongly increased the activation of JNK, which was induced by IL-1 and sorbitol in epithelial cells and by lipopolysaccharides (LPS) in macrophages[72]. The authors proposed that a negative feedback mechanism exists that is mediated by p38α phosphorylation of TAB1, a subunit of the kinase TAK1 (also known as MAP3K7), which functions as a MAP3K upstream of both p38 MAPKs and JNKs and also phosphorylates inhibitor of nuclear factor kappa-B kinase subunit-β (IKKβ), leading to nuclear factor kappa-B (NF-κB) activation[72] (FIG. 2). Recent work has proposed that p38α also negatively regulates the activity of MLK3 (also known as MAP3K11), another MAP3K upstream of JNK[73].

Increased activation of JNK on p38α inhibition has also been observed in mouse models. For example, p38α-deficient myoblasts do not exit the cell cycle and proliferate in differentiation-promoting conditions, which is accounted for by increased activation of the JNK/JUN pathway[64]. This JNK hyperactivation correlates with reduced levels of the MAPK phosphatase dual specificity protein phosphatase 1 (DUSP1; also known as MKP1) in p38α-deficient myoblasts[64] (FIG. 2). Fetal hematopoietic cells and MEFs lacking p38α also show increased proliferation owing to sustained activation of the JNK/JUN pathway. However, JUN-deficient hepatocytes show increased p38α phosphorylation[74], which is responsible for impaired proliferation after partial hepatectomy, suggesting that the JNK/JUN pathway can also affect p38 MAPK activity. Importantly, mice with a liver-specific deletion of p38α are more susceptible to chemically induced liver cancers, and JNK inactivation suppressed the increased proliferation of p38α-deficient hepatocytes and tumour cells[42]. The hyperactivation of the JNK/JUN pathway in fetal hematopoietic cells was proposed to be mediated by the upregulation of the adaptor protein GRB2-related adaptor protein 2 (GRAP2), which in turn leads to the activation of the kinase HPK1 (also known as MAP4K1) (FIG. 2). By contrast, JNK upregulation in p38α-deficient MEFs does not seem to involve GRAP2 or HPK1, but correlates with increased phosphorylation of MKK7 (REF. 42). Treatment of mice with LPS also induces JNK hyperactivation in p38α-deficient livers, which does not seem to depend on JNK phosphatases, but correlates with increased phosphorylation of MKK3, MKK4 and MKK6 (REF. 75). This suggests that a negative feedback loop is involved at the level of an upstream MAP3K, as proposed for TAK1 and MLK3 (REFS 72,73). Therefore, p38α can antagonize JNK signalling by different mechanisms depending on the cell type and stimulus (FIG. 2).

Mouse models of cancer have also provided evidence for the interaction of both JNK and p38 MAPKs with the NF-κB pathway, a key regulator of cell survival and inflammatory processes. Hepatocytes deficient in the NF-κB activator IKKβ show reduced JNK phosphatase activity, which leads to sustained JNK1 activation and increased liver carcinogenesis[76]. However, the high levels of JNK1 activity induced by p38α downregulation are not sufficient to increase the sensitivity of hepatocytes to LPS or TNF-induced toxicity, unless NF-κB is also partially inhibited[75]. The increased LPS-induced liver damage caused by simultaneous inhibition of the p38α and NF-κB pathways correlates with reduced levels of the caspase 8 inhibitor CFLAR[75] (FIG. 2). JNK activation can also be negatively regulated by the NF-κB target gene *Gadd45β*, which has been implicated in the regulation of TNF-induced cell death and liver regeneration[77,78]. These results illustrate how cell fate decisions are regulated *in vivo* by complex interactions between different signalling pathways that are determinants for cancer development (FIG. 2).

Inflammation, migration and metastasis

Chronic inflammation is a potent cancer promoter[79,80], and has been linked to increased cancer cell survival and the induction of angiogenesis and invasion. Evidence for the control of these processes by the JNK and p38 MAPK pathways comes from the fact that these enzymes regulate the activity and expression of key inflammatory mediators, including cytokines and proteases, which affect cancer progression.

Functions of JNKs. The JNK pathway has been linked to the expression of cytokines that control inflammation and cancer, although to a lesser extent than p38 MAPKs[4]. The role of JNKs in liver inflammation and cancer has been studied using the TNFα-mediated concanavalin A mouse model (Con A mouse model) for acute hepatitis, which involves T cells and cytokines. During Con A-mediated hepatitis, binding of TNFα to its receptors results in JNK activation by a complex signalling network, which leads to the induction of AP1 and NF-κB. Con A-mediated hepatitis is prevented in mice that lack JNK1 or JNK2 (REF. 81) and is probably mediated by an ITCH–caspase 8–CFLAR pathway. In contrast to JNK, JUN mediates hepatocyte survival by transcriptionally regulating the expression of inducible nitric oxide synthase (NOS2), controlling the subsequent release of nitric oxide and protecting the liver from hypoxia and oxidative stresses, which are events that are often linked to cancer[82]. Interestingly, inhibition of NF-κB in a mouse model of liver cancer results in sustained JNK activity, increased cell death and cytokine-driven compensatory proliferation[81,83], indicating that both JUN/AP1 and NF-κB functionally antagonize the death-promoting

Concanavalin A mouse model
This hepatitis model requires intravenous injection of the lectin concanavalin A and is dependent on T cells and inflammatory cytokines, such as TNFα. It recapitulates aspects of viral and autoimmune hepatitis in humans.

functions of JNK. Recently, Con A-induced hepatitis was shown not to be affected in mice that lacked both JNK1 and JNK2 in hepatocytes, although hepatitis development was prevented when JNK1 and JNK2 were deleted only in hematopoietic cells[84] (TABLE 1). It was proposed that TNFα production by myeloid cells might be important for JNK1 and JNK2 function, although the liver damage measured by the levels of liver-specific enzymes was 80–90% less than the levels published in other studies.

Recent work has linked the endoplasmic reticulum (ER) stress response to JNK activation in intestinal inflammation, and possibly cancer progression. Mice lacking the transcription factor XBP1 in intestinal epithelial cells develop spontaneous enteritis with increased susceptibility to colitis and inflammatory bowel disease[85]. Interestingly, XBP1 single nucleotide polymorphism variants have been associated with human inflammatory bowel disease, suggesting that increased JNK signalling might link cell-specific ER stress to the induction of organ-specific inflammation and subsequent cancer development. Other inflammatory diseases proposed to be controlled by JNK signalling in mouse models are rheumatoid arthritis, in which JNK1 and JNK2 regulate the expression of metalloproteinases and TNFα[86], and atherosclerosis in which JNK2 is proposed to phosphorylate scavenger receptor A[87]. The link between the expression of JNK-dependent targets, such as metalloproteinases and inflammatory cytokines, and cancer progression has yet to be established in mouse models of cancer and metastasis.

Functions of p38 MAPKs. p38α regulates the induction of the pro-inflammatory mediator cyclooxygenase 2 (COX2), which could potentially contribute to cancer progression in non-melanoma skin cancer, breast cancer and gliomas[88–90]. In addition, p38α has a key role in the production of many cytokines, such as TNFα, IL-1 and IL-6, which have pro-inflammatory, pro-survival and angiogenic effects[91]. p38α can regulate cytokine expression by modulating transcription factors, such as NF-κB[79], or at the post-transcriptional level, by regulating mRNA stability and protein translation, which is thought to be mostly mediated by the downstream kinase MK2 (REF. 92). Specific deletion of p38α in myeloid or epithelial cells has provided *in vivo* evidence for the importance of this pathway in cytokine production and inflammatory responses[93,94] (TABLE 1). By contrast, p38β does not seem to be required for acute or chronic inflammatory responses[95,96].

p38α may also directly affect tumour invasion and angiogenesis independently of its role in inflammation. For example, p38α can induce expression of the matrix metalloproteinases MMP1, MMP3 and MMP13, which regulate matrix remodelling and degradation by metastatic cancer cells, as well as vascular endothelial growth factor A (VEGFA), a potent inducer of tumour survival and angiogenesis[21]. In addition, p38α can activate hypoxia inducible factor 1 (HIF1), which has a key role in hypoxia-driven expression of angiogenic factors, at least partly through the stabilization of its α-subunit[97]. Experiments that used cancer cell lines in various invasion assays further support a role of p38α in metastasis.

Table 1 | **Phenotypes of Jun N-terminal kinase and p38 mitogen-activated protein kinase knockout mice**

MAPK	Phenotype	Disease and cancer model	Refs
MAPK8$^{-/-}$ (JNK1)	Thin epidermis, T cell differentiation defects	Hepatitis resistant (Con A) Liver cancer reduced (DEN) Skin cancer increased (DMBA and TPA) Gastric cancer reduced (MNU)	5,81 112,113 115 121
MAPK9$^{-/-}$ (JNK2)	Keratinocyte hyperplasia, T cell differentiation defects	Hepatitis resistant (Con A) Liver cancer not affected Skin cancer reduced (DMBA and TPA)	5,81 113 116
MAPK10$^{-/-}$ (JNK3)	Resistance to excitotoxic neuronal cell death	Not determined	5
MAPK9$^{-/-}$ *MAPK8 f/f × Alb-Cre*	No phenotype	Hepatitis — no effect (Con A)	84
MAPK9$^{-/-}$ *MAPK8 f/f × Mx-Cre* *MAPK9*$^{-/-}$ *MAPK8 f/f × Fabp4-Cre*	No phenotype Fat phenotype	Hepatitis resistant (Con A) Diabetes, hepatic insulin resistance	84 159
MAPK14$^{-/-}$ (p38α) *MAPK14 f/f × More-Cre* *MAPK14 f/f × RERTn-Cre* *MAPK14 f/f × Alb-Cre*; *Mx-Cre* *MAPK14 f/f × MLC-2a-Cre* *MAPK14 f/f × Lys-Cre* *MAPK14 f/f × K14-Cre*; *Lys-Cre*	Placental defect, death by E12.5 Postnatal death, hyperproliferation of fetal haematopoietic cells Lung hyperplasia Erythroid proliferation defects Proliferation of adult cardiomyocytes Reduced cytokine production Reduced inflammatory responses	Not applicable Not applicable Lung cancer increased (*Kras*^{G12V}) Liver cancer increased (DEN and Pb) Not determined Sepsis (LPS) Skin injury (SDS and UVB)	160–162 42 43 42 41 94 93
MAPK11$^{-/-}$ (p38β) *MAPK12*$^{-/-}$ (p38γ) *MAPK13*$^{-/-}$ (p38δ)	No phenotype No phenotype Impaired insulin secretion and survival of pancreatic β-cells	Not determined Not determined Diabetes Skin cancer (DMBA and TPA) and lung cancer (*Kras*^{G12V}) reduced	95 163 164 44

Con A, concanavalin A; DEN, diethylnitrosamine; DMBA, 12-dimethylbenz(a)anthracene; *f/f*, flox/flox conditional allele; JNK, Jun N-terminal kinase; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MNU, methyl-nitroso-urea; Pb, phenobarbital; SDS, sodium dodecyl sulphate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; UVB, ultraviolet B.

For example, p38α activity is required for the invasive capacity of human hepatocellular carcinoma (HCC) and head and neck squamous cell carcinoma cell lines[98,99], and increased activation of p38α by MKK3 promotes glioma invasiveness both in cell-based assays *in vitro* and in rat brain slices *ex vivo*[100]. In addition, regulation of tumour cell extravasation by p38α has been proposed to account for the reduced number of lung metastases observed on intravenous injection of B16 or LLC tumour cells in p38α heterozygous versus wild-type mice[101]. p38δ has also been proposed to regulate squamous cell carcinoma invasion[99], and p38γ has been associated with Ras-induced invasion[102]. By contrast, the p38 MAPK activator MKK6 has been reported to suppress metastasis[103] or to have no effect[104], depending on the experimental model used.

In addition to facilitating metastasis by the mechanisms discussed above, p38α also regulates cancer cell migration[105–109]. MK2 has an important role in cell migration downstream of p38α by remodelling the actin cytoskeleton. This cytoskeletal remodelling may be mediated by the phosphorylation of heat shock 27 kDa protein (HSP27), inducing its release from F-actin caps[108], or by the activation of the protein kinase LIMK1, which in turn phosphorylates and inactivates the protein cofilin[110].

Mouse cancer models

Genetically engineered mouse models for components of the JNK and p38 MAPK pathways, as well as carcinogenesis studies in various organs, have provided new insights into the diverse and sometimes opposing molecular functions of JNKs and p38 MAPKs (TABLE 1).

Functions of JNKs. The three JNK proteins can exert pro- and anti-oncogenic functions in different cell types and stages of cancer development.

HCC is the third most common cause of death from cancer in the world. Several studies have demonstrated a requirement for JUN in the development of HCC through antagonizing the pro-apoptotic function of p53 (REF. 111). Similarly, JNK1 deficiency (but not JNK2 deficiency) has been shown to significantly decrease susceptibility to diethylnitrosamine (DEN)-induced HCC formation[112] (TABLE 1). Reduced HCC development correlated with decreased expression of cyclin D and VEGFA, as well as reduced hepatocyte proliferation and neovascularization. These findings were recently substantiated, both in mouse models and in human tumour cell lines, showing that impaired liver cell proliferation and tumour formation following JNK1 downregulation is caused by reduced expression of MYC and increased expression of the CDK inhibitor p21 (REF. 113). Pharmacological inhibition of JNKs with an inhibitory peptide also reduced HCC development in both cancers in mice induced by a DEN–phenobarbital protocol and in xenografted human HCC cells, suggesting that JNK1 targeting should be considered as a new therapeutic approach for HCC treatment[114].

The contribution of either JNK1 or JNK2 to tumour development was also investigated in mouse skin carcinogenesis using the DMBA–TPA protocol. In contrast to liver cancer, *JNK1*-knockout mice seemed to be more susceptible to skin tumours, whereas papilloma formation was found to be substantially reduced in *JNK2*-knockout mice[115,116]. These data imply an oncogenic function for JNK2, whereas JNK1 seems to be a suppressor of skin tumour development. At the molecular level, it was suggested that differential regulation of ERK and AKT signalling, as well as altered AP1 DNA binding activities, could account for the specific functions of JNK1 and JNK2 in skin tumours. Whether these differences are the molecular basis for the cell context-dependent JNK phenotypes observed in skin versus liver needs to be investigated in future experiments.

On the basis of studies of Apc^{min} mice and transgenic mice that specifically express JNK in the intestine, JNK1 and JUN have been proposed to be essential mediators of oncogenic β-catenin signalling in tumours of the small intestine[117,118]. By contrast, chemically induced colitis-associated tumour formation was not affected by inactivating JNK1 or JUN in intestinal epithelium and JUN in myeloid cells[119]. Moreover, a study has shown that JNK1-deficient mice spontaneously develop intestinal tumours[120], although this finding could not be confirmed by other laboratories. The exact role of JNK proteins in intestinal tumorigenesis is not fully understood and requires further investigations using conditional alleles of the respective genes.

JNK activation has been detected in human gastric cancer samples and, consistent with this, JNK1 controls both tumour initiation and promotion by affecting cell proliferation and ROS production in a mouse model of gastric cancer caused by methyl-nitroso-urea treatment[121].

The leukaemia-associated BCR–ABL protein activates the JNK pathway in haematopoietic cells, leading to increased AP1 transcriptional activity and inhibition of apoptosis. In addition, disruption of JNK1 inhibits transformation of pre-B cells *in vitro* and *in vivo*; this is partly mediated by the expression of AKT and BCL2 (REF. 122), which is a signalling pathway that is also relevant in other tumours and is amenable to targeted therapies.

Functions of p38 MAPKs. Subcutaneous xenograft experiments have shown that MEFs deficient in either p38α[123] or the p38 MAPK activators MKK3 and MKK6 (REF. 124) lead to a higher oncogene-induced tumour burden in nude mice than their wild-type counterparts. The function of p38α as a tumour suppressor *in vivo* has been substantiated by studies that used genetically modified mice. Protein phosphatase 1D (PPM1D; also known as WIP1) transcription is stimulated by p53 and can target p38 MAPKs, among other substrates. Genetic inactivation of PPM1D reduces mammary gland tumorigenesis in mice that express the *Erbb2* or *Hras* oncogenes, which are under the control of the mouse mammary tumour virus (MMTV) promoter. This correlates with increased levels of p38 MAPK activity and apoptosis. Importantly, a chemical inhibitor of p38α and p38β restores mammary tumour formation in PPM1D-deficient mice that express MMTV-*Erbb2* (REF. 125). Conversely, targeted expression of PPM1D in the breast epithelium increases the sensitivity of mice to MMTV-*ErbB2*-induced mammary tumorigenesis, whereas the co-expression of a

DEN–phenobarbital protocol
A fully established and widely used model for liver cancer development in mice. In several studies only the carcinogen DEN (diethylnitrosamine) is applied to young mice without tumour promotion, whereas the classical protocol depends on two-stage carcinogenesis with DEN being applied as the initiator and phenobarbital as the promoter of liver carcinogenesis.

DMBA–TPA protocol
A widely applied and fully established two-stage skin carcinogenesis protocol that depends on tumour initiation with DMBA (7,12-dimethylbenz(a)anthracene) and promotion with TPA (12-O-tetradecanoylphorbol-13-acetate).

Apc^{min} mice
Mice carrying a mutation in the *Apc* tumour suppressor gene, which is thought to initiate intestinal tumorigenesis. Mutation of *Apc* leads to increased β-catenin-mediated transcription of proliferation-promoting genes.

constitutively active form of the p38 MAPK activator MKK6 in the mammary gland abolishes the effect of PPM1D overexpression[126]. Studies in mice deficient for GADD45α, an activator of MEKK4 that functions as a MAP3K upstream of both p38 MAPKs and JNKs (FIG. 1), also support a tumour suppressor role for these pathways in breast cancer. Deletion of *Gadd45α* increases MMTV-Ras-induced mammary tumour development, which correlates with reduced levels of both apoptosis and senescence, probably owing to the impaired activation of JNK and p38 MAPKs, respectively[127]. The activation of p38α has also been linked to liver cell apoptosis in mouse models that either overexpress the TGFβ signal transducer *Smad3* (REF. 128) or have impaired ATF2 transcriptional activity[129].

More direct evidence for the negative regulation of tumorigenesis by p38 MAPKs in mice has been provided by studies that used conditional p38α alleles (TABLE 1). p38α-deficient mice are sensitized to *Kras*-induced lung tumorigenesis, which has been attributed to the immature and hyperproliferative lung epithelium that results from p38α inactivation[43]. In addition, hepatocyte-specific deletion of p38α promotes chemically induced liver cancer, for which upregulation of the JNK/JUN pathway plays an important part in increased proliferation of p38α-deficient tumour cells[42]. A recent report has shown that p38α suppresses ROS accumulation and cell death in hepatocytes, and it was proposed that the release of IL-1α by necrotic hepatocytes may indirectly contribute to liver carcinogenesis by stimulating carcinogen-induced compensatory proliferation[130]. However, mice that lack p38δ show reduced susceptibility to the development of both skin carcinomas induced by DBMA and TPA treatment and *Kras*-induced lung tumours, suggesting that p38δ positively regulates tumorigenesis[44].

Finally, deletion of the protein kinase MK5 (also known as PRAK or MAPK5) increases skin carcinogenesis induced by DBMA in the absence of any tumour promoter, and also accelerates the lymphomagenesis induced by the expression of an *Eμ-NRas*^{G12D} transgene[131]. These results suggest that MK5 may act as a tumour suppressor; its function is probably mediated by the regulation of oncogene-induced senescence, although the extent of the p38 MAPK contribution to MK5 activation is controversial[132]. Therefore, more mechanistic studies are required to define the functions of p38 MAPKs, their regulators and targets in mouse models of cancer.

JNK and p38 MAPK pathways in human cancer

Altered expression of JNK and p38 MAPK proteins in human tumours and cancer cell lines is often observed, although it is rarely known whether these proteins are causally involved in proliferation control and the development of a specific tumour type. The best evidence for a role of these pathways in human cancer comes from genetic studies identifying MKK4, a MAP2K that activates both JNKs and p38α (FIG. 1), as a putative tumour suppressor. Loss-of-function alleles were found in 5% of human pancreatic, lung, breast, colon and prostate cancer, although there is also evidence from *in vitro* studies that MKK4 can promote tumorigenesis[133,134]. The emerging views on how these pathways might affect human cancers, as well as evidence from human genetic studies, are discussed below and are summarized in TABLE 2.

JNKs and human cancer. Large-scale sequencing analyses of protein kinases in human tumours have identified somatic mutations in the JNK pathway, that presumably activate JNK1, JNK2 and the upstream kinase MKK7 (REFS 135,136). These data suggest that mutations in the JNK pathway can be involved in cancer development.

As in rodent model systems, in many human cancers, JNKs can exert dual functions, either oncogenic or tumour suppressive. In HCC, JNK1 seems to be oncogenic, as increased kinase activity and proliferation of tumour cells are correlated with an increase in tumours[113] (TABLE 2). Moreover, sustained JNK1 activation was found to be associated with altered histone H3 methylation in human HCCs[137]. Interestingly, as for MKK4, JNKs are also mutated in cancer and the loss of JNK3 function promotes tumour formation. For example, *MAPK10* (which encodes JNK3) might be a putative tumour suppressor gene, as mutations were found in 10 of 19 human brain tumours examined[138].

The role of JNKs in prostate cancer development is of particular interest, because this cancer is one of the most common neoplasms in ageing males and is a serious health problem. The tumour suppressor PTEN is the second most commonly mutated tumour suppressor in human cancers and is frequently lost in prostate cancer. PTEN loss leads to AKT activation and increased JNK activity in various human cancer cell lines and human clinical prostate cancer samples[139]. Gene expression analyses were used to show that several members of the JNK pathway were upregulated in prostate cancer[140], whereas JUNB was identified as an inhibitor of prostate carcinogenesis[141]. Furthermore, PTEN deficiency sensitizes

Table 2 | **Role of Jun N-terminal kinases and p38 mitogen-activated protein kinases in human cancer**

Human cancer	MAPK implication	Refs
Liver cancer (hepatocellular carcinoma)	JNK1 highly activated p38α activity downregulated	112,113,137 145
Brain tumours	JNK3 loss-of-function mutations	138
Prostate cancer	JNK1 upregulated	139,140
Lymphoma, glioma, lung, thyroid, breast, head and neck squamous cell carcinomas	p38α activity upregulated	99,100, 146–149

JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

Table 3 | **Inhibitors of Jun N-terminal kinases and p38 mitogen-activated protein kinases in clinical trials**

Compound	Primary target	Clinical trials	Sponsor
JNK 930 (CC-930)	JNK	Phase I: fibrotic diseases, inflammatory disease	Celgene Corporation
JNK 401 (CC-401)	JNK	Phase I/II: myelogenous leukemia (discontinued)	Celgene Corporation
Semapimod	p38 MAPK, JNK, MEK	Phase II: Crohn's disease, cancer, HIV, inflammatory disease	Cytokine PharmaSci
XG-102 (D-JNKI-1) (TAT-coupled peptide)	JNK	Phase II: stroke, Alzheimer's disease	Xigen
Talmapimod (SCIO-469)	p38 MAPK	Phase I/II: multiple myeloma, myelodysplastic syndromes, pain	Scios
VX-702	p38 MAPK	Phase II: rheumatoid arthritis	Vertex
SB-681323	p38 MAPK	Phase I/II: rheumatoid arthritis, neuropathic pain	GlaxoSmithKline
ARRY-614	p38 MAPK, TIE2	Phase I: haematological cancers	Array BioPharma
ARRY-797	p38 MAPK	Phase I/II: dental inflammatory pain, cancer	Array BioPharma
PH-797804	p38 MAPK	Phase II: arthritis, pain	Pfizer

JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

cells to JNK inhibition and therefore JNKs can be seen as crucial components of the PTEN–PI3K pathway and as potential therapeutic targets.

Recently, analysis of *Drosophila melanogaster* segmentation networks identified the E3 ubiquitin ligase complex factor SPOP as an important regulator of JNK activity, which seems to be conserved in TNF-mediated JNK signalling. Moreover, SPOP was found to be overexpressed in 99% of clear cell renal carcinomas, the most prevalent form of kidney cancer[142]. This study illustrates the power of the use of genetic studies for the identification of new cancer genes.

p38 MAPKs and human cancer. In support of the tumour suppressor function of p38α, several negative regulators of p38 MAPK signalling have been found to be overexpressed in human tumours and cancer cell lines, including the phosphatases PPM1D and DUSP26 (REFS 123,143,144) and the inhibitors of the MAP3K apoptosis signal-regulating kinase 1 (ASK1), glutathione *S*-transferase Mu 1 (GSTM1) and GSTM2 (REF. 52). Large-scale sequencing has also identified somatic mutations in the p38 MAPK pathway in a small number of human tumours[135], but the importance of these mutations remains to be investigated. Interestingly, some human tumours, such as HCCs, have lower p38 MAPK and MKK6 activity than non-tumorigenic tissues (TABLE 2), supporting the observation that increased p38 MAPK activity induces apoptosis in hepatoma cell lines[145]. However, increased levels of phosphorylated p38α have been correlated with malignancy in various cancers, including follicular lymphoma, lung, thyroid and breast carcinomas, as well as glioma and head and neck squamous cell carcinomas[99,100,146–149] (TABLE 2). These observations are consistent with the idea that, depending on the type of cancer and tumour stage, cancer cells with pro-tumorigenic mutations in the p38 MAPK pathway have a selective advantage.

In summary, these data imply that inhibition of the JNK and p38 MAPK pathways for therapeutic purposes should be strictly dependent on cell context, tumour cell type and tumour stage.

p38 MAPK and JNK as drug targets

The key roles of p38α in the production of pro-inflammatory cytokines and in the signal relay from cytokine receptors have led to the development of a large number of small-molecule p38 MAPK inhibitors (TABLE 3). Many of these have shown high specificity towards p38α *in vitro* and have yielded promising results in preclinical studies for inflammatory diseases, but few have progressed beyond Phase I clinical trials, mostly owing to side effects, such as liver toxicity[91,150]. However, it is unclear whether the side effects are due to p38 MAPK inhibition or to the drugs that act on additional targets. A new generation of more selective p38 MAPK inhibitors is currently being developed that seem to have less toxic effects in animal models. An important consideration, given the evidence in support of p38α as a tumour suppressor[20,21], is whether inhibition of p38α might result in an increased predisposition to cancer. This possibility was supported by experiments in mouse models of lung and liver cancer[42,43]. However, chronic inflammation is a potent cancer promotor and cytokines are important survival factors for cancer cells[79,80], suggesting that p38α inhibition might be beneficial for the treatment of inflammation-associated cancers, such as colon cancer and possibly HCC.

The p38α inhibitors may also be useful to treat tumours that rely on p38 MAPK activity for progression, or could be combined with DNA-damaging chemotherapy to trigger cancer cell death by impairing p38α-mediated cell cycle arrest and DNA repair mechanisms[100,151]. In addition, p38 MAPK inhibition may increase the sensitivity of cancer cells to the effect of chemotherapeutic drugs that usually act on dividing cells. For example, quiescent leukaemic cells treated with a p38 MAPK inhibitor proliferate and become sensitive to the effect of the anti-mitotic drug 5-fluorouracil[152]. As a note of caution, combination therapies should take into account that many chemotherapeutic drugs require a functional p38α pathway for efficient action[153]. Finally, drug-induced activation of p38α, for example by inhibiting phosphatases, could be a strategy worth

exploring for sensitizing cancer cells to apoptotic death. However, this might induce an inflammatory response as a side effect, which results in increased angiogenesis and invasion. Given the many tumorigenesis-related functions that p38α can control, both in the cancer cell and in the tumour microenvironment, it is important to carefully consider both the type and tumour stage before attempting to modulate p38α activity for cancer therapy.

The diverse functions of JNKs on cell proliferation and on the induction of cell death are also being explored for targeted therapies that use both peptide inhibitors and small molecules[154]. Many of the above considerations for p38α also apply to JNK-dependent targeted therapies. Several companies have JNK inhibitors with different core structures at different stages of development (TABLE 3). Many inhibitors, such as SP-600125, lack specificity and selectivity for the different JNK isoforms[155], although a recent report describes a JNK1-specific inhibitor[156]. However, several peptide inhibitors have been developed, such as JNKI1 (REF. 157), which has been successfully used in mouse models, for example, for HCC[113], or BI-78D3, which inhibits JNK activity through interfering with binding to the JNK-interacting protein 1 (JIP1) scaffold[158]. As the field of JNK inhibitors is rapidly moving, it is anticipated that several targeted therapies with new drugs will be successfully applied and used in the clinic in the near future.

Conclusions and perspectives

Will future therapeutic strategies depend on new mouse models and human genetic studies? One of the lessons learned from mouse genetic studies is that specific side effects can be observed by targeting JNKs and p38 MAPKs. For example, the increased lung tumorigenesis observed in p38α knockout mice strongly suggests that p38α inhibitors should not be given to people with an increased risk of developing lung cancer. Similarly, the increased liver cancer development in p38α-deficient mice should also be considered. These studies, as well as the analysis of human cell lines and tumours, should provide important information on which types of cancer are likely to respond to therapies targeted against JNKs and p38 MAPKs.

Another lesson learned from analysing mouse models is the existence of crosstalk between the MAPK signalling pathways in different cell types and stages of cancers. Given the complexity of the underlying molecular mechanisms, inhibiting these pathways is a big challenge, which should nevertheless be undertaken with selective drugs. Whether targeting particular JNK and p38 MAPK genes or isoforms will be more beneficial than targeting the whole pathway must be considered. Moreover, combination therapies are likely to be important in the future, although deciding which drugs should be combined is not trivial. It is therefore essential to mechanistically understand the functions of MAPK family members in different tumour types and how the interplay between them and interactions with other signalling pathways are wired.

Genomic and proteomic strategies will certainly identify new MAPK substrates and regulators, whereas molecular signatures obtained from sequencing and array data in response to drug treatments should lead to the identification of both new biomarkers and potential targets for therapies. However, which targets will be clinically relevant and allow the eventual treatment of cancer patients? To better validate these targets, human transplantation and mouse tumour models are essential. Mouse genetic studies are time-consuming and human xenografts are rightly considered artificial. However, mouse cancer models are continuously evolving to better resemble the pathogenesis of human cancer and although they are demanding, both in terms of time and resources, they provide valuable information in addition to *ex vivo* experiments with cultured cells. One alternative experimental strategy is the treatment of freshly dispersed primary tumours with new drugs, followed by transplantation into immune-deficient mice, which provides a rapid initial efficacy test.

The final goals of studies using mouse models and human genetic and pharmacological approaches are to better define the specific roles of MAPK signalling in specific tumours. In addition to angiogenesis, future therapies should target the microenvironment and other non-tumorigenic cells, such as macrophages and stromal cells. We are convinced that promising new avenues for the treatment of cancer are on the horizon, which will undoubtedly lead to better, more efficient and faster therapies in the years to come.

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Acknowledgements
We are grateful to L. Bakiri, L. Hui, N. Kraut, K. Sabapathy and M. Thomsen for critical comments on the manuscript and A. Bozec for help with the figures. Work in the laboratory of E.F.W. and A.R.N. is funded by the Centro Nacional de Investigaciones Oncológicas. A.R.N. is also supported by grants from the Spanish Ministerio de Ciencia e Innovación (MICINN) (BFU2007-60575), Fundación La Caixa, MICINN/ISCIII-RTICC RD06/0020/0083 and the European Commission FP7 programme grant 'INFLA-CARE' (EC contract number 223151), and E.F.W. is also supported by the consortium CELLS INTO ORGANS of the EC-FP7 and the BBVA-Foundation.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
HRAS | *MAPK8* | *MAPK9* | *MAPK10* | *MAPK11* | *MAPK12* | *MAPK13* | *MAPK14* |
UniProtKB: http://www.uniprot.org
ATF2 | BAD | BAX | caspase 8 | C/EBPβ| CFLAR | E47 | EGFR | ELK1 | ERK1 | ERK2 | JNK1 | JNK2 | ITCH | MK2 | MKK3 | MKK4 | MKK6 | MKK7 | MNK1 | MNK2 | MSK1 | p38α | p38β | p38δ | p38γ | p53 | PPM1D | TNFα

FURTHER INFORMATION

Erwin F. Wagner's homepage: http://www.cnio.es/ing/grupos/plantillas/presentacion.asp?grupo=50006422
Ángel R. Nebreda's homepage: http://www.cnio.es/ing/grupos/plantillas/presentacion.asp?grupo=50004261

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