p38 mitogen-activated protein kinases: their role in carcinogenesis

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The members of the p38 subfamily of Mitogen-Activated Protein Kinases (MAPKs) are a versatile group of proteins, that function as signal transducers involved in key cellular functions. Initially, p38 MAPKs were associated with inflammatory and cellular stress responses and, as such, p38 has been an important target for anti-inflammatory therapies. Recently, increasing evidence has directly implicated p38 MAPKs in processes essential for cell development, transformation and tumorigenesis, namely: proliferation, differentiation, apoptosis, invasion and metastasis. These features make p38 MAPKs potential targets for future anti-neoplastic therapies.

Key words: p38, MAP kinases, serine-threonine kinases, carcinogenesis, transformation.

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Participación de MAP quinasas p38 en carcinogénesis

Los componentes de la subfamilia p38 de quinasas activadas por mitógenos (MAP quinasas) constituyen un grupo de proteínas muy versátiles, que actúan como transductoras de señales en funciones celulares de capital importancia. Originalmente las proteínas p38 han sido asociadas con respuestas inflamatorias y de estrés celular, debido a lo cual p38 se ha convertido en una diana terapéutica de especial relevancia en el control de enfermedades asociadas a estos procesos.

No obstante, actualmente existen evidencias considerables para también implicar directamente a p38 en eventos de capital importancia dentro de la transformación celular y la tumorigénesis, en concreto: proliferación, diferenciación, apoptosis, invasión y metástasis. Estas propiedades de las MAP quinasas p38 las convierten en potenciales dianas para futuras terapias antineoplásicas.

Palabras clave: p38, MAP quinasas, serina-threonina quinasas, carcinogénesis, transformación.

Mitogen-activated protein kinases (MAPKs) are cytoplasmic serine/threonine kinases that are activated in response to a wide array of extracellular stimuli, including those that regulate cell proliferation, differentiation, development and inflammation. MAPKs are pivotal elements in the transduction of signals from the membrane to the interior of the cell, acting as essential mediators in signalling modules that include sequentially: MAP kinase kinases (MAPKKs), dual-specificity MAP kinase kinases (MAPKKs) and MAPKs. To date, four mammalian MAPKs families have been studied in detail: ERKs (extracellular signal

regulated kinases), JNK/SAPKs (c-jun N-terminal kinase/stress activated protein kinases), ERK5/BMKs (Big MAP kinases) and p38 MAPKs^{1,2}. Even though the activation of the different MAPKs was originally associated to defined types of stimuli, it is now clear that most stimuli can switch on several MAPK modules and that the biological outcomes of such stimuli are consequences of the integration of the intensity and duration of the signals transduced by each individual MAPK route. The biological mechanisms involved in cellular transformation and oncogenesis are no exception and, to date, all MAPKs have been found to be involved, one way or another, in distinct aspect of carcinogenesis. This review will focus in the role played by p38 MAPKs in those processes that turn awry at some stage of the malignification process.

THE p38 MAPK FAMILY

p38 α /CSBP2/RK2 was first identified as an endotoxin-stimulated kinase³. At the same time, a target for imidazole anti-inflammatory drugs was identified,

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TABLE 1. Main features of p38 family members

Member	Identification	Splicing isoforms	Tisular distribution	Imidazole response	Activating stimuli	Substrates	Special features
p38 α CSBP2 RK2	CSBP2 had great homology with yeast protein HOG. It was identified by several approaches ⁴	A 25 residue internal sequence makes CSBP1 different from CSBP2. CSBP1 complements HOG 1 deletion in yeast. Mxi2 ¹² and Exip ¹³ are two other alternative splicing products	p38 α is ubiquitous and the rest of the isoforms present a more restricted pattern of expression. Mxi2 appears to be only expressed in kidney ²⁴	CSPB2 activity is inhibited by imidazoles, but Mxi2 isn't ²⁵	This kinase is activated by IL-1 β , TNF- α , sorbitol (osmotic shock) or UV radiation. Both MKK3 and MKK6 can activate it ²	It phosphorylates MBP, ATF-2, MAPKAP-K2, MAPKAP-K3, MNK, MSK, CHOP, PRAK, MEF2C and Max, but not c-Jun, as the rest of p38 family members. Sap-1a y Elk-1 are also good substrates ¹⁶⁻²³	CSBP2 is the most abundant isoform of the family and has been more extensively studied
p38 β	In 1996 Jiang et al described the cloning and characterization of this family member with 74% homology with p38 α	Kumar et al tried to clone p38 β by RT-PCR using Jiang et al sequence, but the cDNA obtained had an internal deletion of 8 $\alpha\alpha$, which they named p38 β 2 ⁷	Similar pattern of distribution as p38 α , being expressed in kidney, pancreas, muscle, spleen, lung, placenta, heart and brain	It's kinase activity is inhibited by imidazole compounds ⁵	It is activated by the same kind of stimuli as p38 α , showing a slower activation kinetics. It is not activated by phorbol esters. MKK6 can activate it although MKK3 cannot ⁵	MBP, ATF-2, MAPKAP-Kinase 2, MAPKAP-Kinase 3 are good substrates, but not c-Jun ⁷	The extra and unique 8 $\alpha\alpha$ p38 β are an insertion when comparing with the rest of MAPKs, so p38 β 2 has more correlated sequence when compared with the other family members ⁷
p382	In 1997, Stein et al described a new isoform, p382, identical to p38 β except in 3 aminoacids ¹⁴	This isoform was isolated in three different forms, two of them have no phosphorylation motif	It's expression pattern differs from p38 α , being most abundant in heart and testes and less abundant in placenta and ovaries ¹⁴	It's activity can be inhibited by imidazoles as much as p38 α	Stein et al showed that anisomycin, UV and sorbitol activated this kinase. MKK6 is also a good activator.	It can phosphorylate ATF-2, Elk-1 and Sap-1a.	It's remarkable that ATF-2 is 180 times better substrate for p382 than for p38 α ¹⁴
p38 γ SAPK4	Han et al described this kinase in 1996 after identifying it in Gen Bank. It's 63% homologous to p38 α and 62% to p38 β	Not described so far	mRNA of this isoform is just found in skeletal muscle, which suggests a specific function in this tissue ¹⁰	Kumar et al found that this kinase cannot be inhibited by imidazoles, due to mutations in aminoacids 106, 107 and 108 with respect to p38 α ⁷	It's activated by typical stress stimuli, such as IL-1, TNF- α , sorbitol, UV and it is also activated by PMA. It's not regulated by insulin, serum and okadaic acid and it is activated by ortovanadate ⁷	It can phosphorylate ATF-2, and MAPKAP K3, but it is unable to phosphorylate MAPKAP K2 ¹⁰	Lechner et al showed that this isoform works as a signal transducer during differentiation from myoblasts to myotubes in C2C12 cells. This kinase favours nuclear myotubular fusion rate, which is a sign of differentiation ²⁶
p38 δ ERK6	In 1997, Jiang et al cloned and characterized this member. This isoform is 60% homologous to p38 α ⁶	They haven't been described	The study of its mRNA revealed a higher expression in kidney and lung ⁶	Kumar et al showed that this kinase is not inhibited by imidazoles ⁷	It's activated by: anisomycin, PMA, arsenite, PAF, oxidative stress, TNF- α , IL-1, IL-6 and UV. Neither serum, insulin, or EGF have any effect on its activity ⁶	It can phosphorylate MBP, ATF-2, Elk-1 and MAPKAP K 3, but not MAPKAPK 2 ⁶	It seems that p38 and p38 δ have evolved towards a different subclass of p38 MAPK which differ from p38 α and from p38 β , having a more restricted tisular distribution, Greater susceptibility to PMA, A different substrate specificity, and a different sensibility to imidazoles ⁶ .

that actually turned up to be p38 α ⁴. Since then, other three p38 isoforms have been described: p38, β , γ /SAPK4, and δ /ERK6⁵⁻¹⁰. Also, splicing variants of p38 α and p38 β , namely CSBP1, Mxi2, Exip¹¹⁻¹³, p38-2 and p38 β 2 have been reported^{7,14}.

p38 MAPKs are substrates for the dual-specificity kinases MKK3 and MKK6 and to a lesser extent for MKK4. Likewise, p38 MAPKs are activated by an ever-growing number of upstream MAPKKs and MAPKKKs². Also, in a fashion similar to SAPK/JNKs, the cascade that leads to p38 activation is regulated at its origins by Rho family GTPases¹.

Once activated, p38 MAPKs can phosphorylate a number of cytoplasmic proteins like cPLA2¹⁵ and kinases like MAPKAPK 2/3¹⁶ MSK¹⁷, MNK¹⁸ and PRAK¹⁹. In addition, p38 MAPKs translocate to the nucleus where they phosphorylate a broad range of transcription factors like those of the Ets²⁰, ATF²¹, MEF²² and GADD²³ families among others. The activation of these downstream mediators sets in motion the cellular processes and genetic programs ultimately responsible for the biological outputs regulated by p38 MAPKs. The main features of p38 isoforms are summarized in table 1.

p38 MAPKS AND CANCER

Even though p38 MAPKs cannot be considered oncogenes *sensu stricto*, as they cannot induce cellular transformation, mounting evidence associate the activation of the p38 pathway to many aspects of carcinogenesis. With respect to MAPKs, oncogenic transformation and tumour progression has been traditionally linked to the aberrant function of the Ras/ERK pathway²⁷. However, it is becoming clear that JNK and p38 MAPKs also play an important role in transformation under some circumstances. A clear case is exemplified by the oncogene *mas* encoding for a G protein-coupled receptor that induces transformation mediated by the activation of the JNK/p38 cascades but with no concomitant activation of ERK²⁸. Another example is the herpes virus associated to Kaposi's Sarcoma HHV8, that also expresses a G-coupled receptor capable of activating both JNK and p38 and provokes cellular transformation and activates angiogenesis²⁹.

Constitutive activation of the p38 pathway can be detected in different types of tumours. Some studies reported high levels of p38 (p38 refers to p38 α hereafter, unless otherwise indicated) kinase activity in non-small cell lung carcinomas³⁰. In human breast tumours analyzed for the expression of p21-activated kinase (PAK), p38 and MAPKAPK-2, all these proteins were overexpressed and exhibited increased activity³¹. Activation of p38 was significantly elevated in well-differentiated prostatic tumours. Furthermore, prostatic intraepithelial neoplastic lesions exhibiting activa-

ted p38, were observed to be proliferative rather than apoptotic³². p38 also appears to be determinant for the progression of multiple myelomas³³. Also, in a cell culture model of ovarian epithelium, activation of signalling proteins, particularly those of the MEK6/p38/MAPKAPK-2 pathway and the PI3K pathway, were found to correlate with phenotypic manifestations of progressive stages in the development of ovarian cancer³⁴. All these cases clearly associate p38 to malignancies. Something that should come as no surprise, as in these past years evidence has mounted relating p38 activation to many essential processes in the up-bringing of cellular transformation and tumoral progression.

p38 IN APOPTOSIS/CELL SURVIVAL

Apoptosis is one of the main safeguards against uncontrolled cell proliferation, by getting rid of those cells that are no longer useful. Apoptosis can be promoted by multiple intra and extracellular signals such as DNA damage, deprivation of growth factors or activation of death receptors³⁵. p38 activation has been associated to apoptosis caused by diverse stimuli in many cellular models²⁷. In diverse cell types, overexpression of upstream components of the p38 cascade is sufficient to induce apoptosis. Fibroblasts transfected with activated MEKK1 die by apoptosis³⁶ and T cells expressing activated Cdc42 have increased JNK/p38 activation and undergo apoptosis due to augmented caspase activity³⁷. ASK1 (apoptosis signal kinase1) can activate p38 direct activators MKK4 and MKK3/6 and when overexpressed, is a potent inducer of cell death³⁸.

In these cases apoptosis induction required massive activation of p38, that may not be reminiscent of a physiological situation. However, a large body of evidence indicates that, at physiological levels, most apoptogenic stimuli are accompanied by p38 activation. Ceramide mediates in apoptosis induction responding to diverse stimuli including TNF, TGF and Fas-Ligand²⁷. p38 activation seems to be a key event in this process since chemical inhibitors for p38 block apoptosis induced by ceramides³⁹. Apoptosis induced by heat shock is also associated with JNK/p38 activation. High levels of heat shock protein Hsp70, protects against cell death, partly by inhibiting JNK/p38 activation⁴⁰.

On the other hand, anti apoptotic stimuli and agents tend to prevent p38 activation. Insulin has neuroprotective effects in foetal neurons. In these cells, insulin specifically inhibits p38 activity⁴¹. In a similar fashion, UV light-induced apoptosis can be blocked by the expression of the dual specificity phosphatase MKP-1 which decreases JNK/p38 activation⁴². Suppression of serum deprivation-induced apoptosis in NIH3T3 by TGF- β is also exerted through the inhibition of p38⁴³.

Likewise, expression of dominant negative MKK3 blocks cisplatin-induced apoptosis⁴⁴.

Although p38 was initially described as an apoptosis-inducing kinase⁵⁸, recent evidence show that depending on the cell type and the apoptogenic stimuli, p38 can act, not only as positive regulator of cell death, but also as a negative one. On one hand, in PC12 cells, p38 activation favours NGF deprivation-induced apoptosis⁴⁵. On other cell types like cardiomyocytes, p38 activation can protect from apoptosis induced by anisomycin⁴⁶. Likewise, in endothelial cells where TNF- α produces inflammation rather than apoptosis, TNF stimulates p38 with bimodal kinetics, interruption of early p38 activation can enhance TNF- α -induced apoptosis, suggesting a protective role for p38⁴⁷. Moreover, the activation of the p38 substrate MEF2C, is necessary for neuron survival, unveiling a direct link between survival and p38 activation⁴⁸. Adding more complexity to the regulation of pro and anti apoptotic functions by p38 family members, there are some studies that provide evidence for differences between p38 isoforms when regulating apoptotic processes. In cardiomyocytes and Hela cells, p38 α induces apoptosis while p38 β promotes cell survival⁴⁹. Furthermore, p38 β but not p38 α can protect mesangial cells from TNF α -induced apoptosis⁵⁰.

Currently, the prevailing line of thought is that, rather than the activation of a single, discrete route, what ultimately determines whether a cell survives or undergoes apoptosis is the dynamic balance between survival and stress signals. The first evidences on this respect, were provided by Xia et al, that found that in PC12 cells the induction of apoptosis depended on the relative contributions of JNK/p38 and ERK activities. In these cells, apoptosis caused by NGF withdrawal was accompanied by a sustained activation of JNK/p38 and the inhibition of ERK⁵¹. A similar case was observed in sympathetic neurons overexpressing MEKK1. Once again, tilting the balance of relative activities towards p38/JNK in comparison to ERKs, resulted in apoptosis⁵¹. On the other hand, transient activation of ERK induced by EGF stimulated proliferation, whereas prolonged ERK activation by NGF induced neuronal differentiation, in both cases JNK/p38 activity levels were low^{52,53}. An analogous situation was observed in the case of constitutively active ASK1, which potentiates apoptosis in NGF-differentiated PC12 cells and rat sympathetic neurons, while a dominant negative ASK1 reduces apoptosis. However, in undifferentiated PC12 cells, moderate expression of ASK1 induces neural differentiation through the activation of p38^{54,55}. These results suggest that a strong activation of ASK-p38 pathway leads to apoptosis, whereas a milder activation would lead to differentiation. These situations also illustrate that the (relative) activation intensity of a MAPK route is not the sole factor in the decision of cell fate. In addition, the du-

ration of the signal is also determinant. As such, in PC12 cells, early/transient and late/sustained activation of JNK/p38 induced by TNF- α have been reported to correlate with survival/differentiation or with apoptosis, respectively^{56,57}.

This interplay among survival pathways, like ERK, and pro-apoptotic pathways such as p38, is highlighted by many anti tumoral drugs, whose mode of action is the induction of apoptosis in the targeted cells. As such, flavopiridol induces apoptosis in chronic lymphocytic leukaemia B-cells by activating p38 and suppressing ERK activity⁵⁸. An identical situation is observed in leukemic U937 cells induced to undergo apoptosis by arsenic trioxide⁵⁹. Lastly, taxol ability to induce apoptosis in different cell types is intimately dependent on its capacity to activate p38 and suppress ERK^{60,61}.

One important question is: how does p38 activation set in motion the machinery responsible for executing apoptosis? Even though this issue remains largely unanswered, the emerging picture, somewhat expected, is that there is not a single culprit. As such in renal cells, p38 mediates cell growth arrest and apoptosis through the transactivation of GADD45 and GADD153 (Growth Arrest and DNA Damage inducible gene)⁶². In response to UV light, p38 stimulates the transcriptional activity of p53⁶³, p38 also regulates the activation of p53 in response to genotoxic stress and inhibition of p38 reduces p53-mediated apoptosis induced by chemotherapeutic agents⁶⁴. In chondrocytes, nitric oxide-induced apoptosis is dependent on p38 activation, that induces the accumulation of p53 via NF- κ B-dependent transcription and its stabilization by direct phosphorylation⁶⁵. A similar situation is observed in UV-induced apoptosis in keratinocytes⁶⁶. An inhibitor of cell cycle progression, the p53-inducible phosphatase Wip1 mediates a negative feedback regulation on p38 signalling in response to UV, by dephosphorylating p38⁶⁷. Inactivation of p38 by Wip-1 facilitates tumour formation⁶⁸. Accordingly, there are recent reports showing that Wip1 is overexpressed in breast tumours⁶⁹.

Other mechanisms are also known to intervene in p38-mediated apoptosis: ASK1 was reported to execute apoptosis mainly by mitochondria-dependent caspase activation⁷⁰. In this context, a large body of data suggests that p38 activation results in cytochrome C release from mitochondria and subsequent activation of caspases 3 and 8^{71,72}, that can be specifically blocked by p38 pharmacological inhibitors⁷³⁻⁷⁶. In light of all these data it becomes evident how by altering p38 activation status some cells may subvert pro-apoptotic mechanisms as a route to immortalization and tumoral growth.

p38 MAPKS IN DIFFERENTIATION

It is well established that apoptosis, proliferation and differentiation are intimately linked processes gover-

ned by common mechanisms. As such, growing evidence also involve p38 in differentiation processes in various cellular systems:

1) Adipocytic differentiation. In 3T3 L1 fibroblasts adipocytic differentiation induced by insulin was blocked by p38 inhibitory mutants or by the p38 specific inhibitor SB203580. Conversely, expression of a constitutively active mutant of MKK6 induced adipocytic differentiation⁷⁷. The transcription factors CCAAT/enhancer-binding protein β (C/EBP β) and peroxisome proliferator-activated receptor (PPAR γ) might be the likely p38 targets during adipogenesis. p38 inhibitors strongly reduce the expression of PPAR γ and the amount of C/EBP β ⁷⁸. Furthermore, it has been demonstrated that p38 inhibitors block the differentiation of mesenchymal C3H10T1/2 cells into adipocytes induced by bone morphogenetic protein 2 (BMP2), essentially by preventing the transcriptional activation of PPAR γ ⁷⁹.

2) Myogenic differentiation. In C2C12 cells, deprivation of growth factors results in myogenic differentiation, that correlates with elevated p38 activity⁸⁰. Moreover, treatment with SB203580 retards myoblast elongation and alignment, prevents myoblast fusion to form multinucleated myotubules, and inhibits the expression of muscle-specific genes. p38 regulates MyoD, a transcriptional activator essential for myogenesis, by two mechanisms: augmenting its levels by increasing its transcription, regulated by the p38 substrate MEF2C, and by increasing its stability through direct phosphorylation⁸¹. It has also been described how activation of p38 induces cell cycle arrest via the inhibition of Raf/ERK pathway during muscle differentiation in myoblasts, by as yet unknown mechanisms⁸².

3) Cardiac hypertrophy is an adaptative response of the heart to a variety of intrinsic and extrinsic stimuli. The hypertrophic response, during which cardiomyocytes increase in size without undergoing cell division, initially serves to compensate for decreased cardiac output; however, prolonged hypertrophy can become detrimental, resulting in dilated cardiomyopathy and heart failure⁹¹. p38 activity was significantly increased in mouse hearts after chronic aortic constriction, coincident with the onset of ventricular hypertrophy⁹². Infection of cardiomyocytes with adenoviruses expressing, MKK3 and MKK6 elicited characteristic hypertrophic responses, including an increase in cell size, enhanced sarcomeric organization, and elevated atrial natriuretic factor (ANF) expression. The direct involvement of p38 in cardiac hypertrophy suggests a significant role for p38 in the pathophysiology of heart failure.

4) Neuronal differentiation. Treatment of PC12 cells with SB203580 or transfection with p38/MKK6 inhibitory mutants originate a profound inhibition of NGF-induced neurite outgrowth⁸³. Conversely, pre-

vious reports had suggested that sustained activation of the ERK pathway was sufficient for PC12 differentiation. This was based on the induction of differentiation, in the absence of NGF, by expression of a constitutively active mutant of MEK1⁸⁴. It now appears that expression of constitutively active MEK1 can also activate p38 and that SB203580 can block MEK1-induced neurite outgrowth, even though ERK would still be active. However, sustained activation of p38 is not very efficient for inducing neurite outgrowth in PC12 cells, except when combined with EGF stimulation, which induces transient activation of both ERK and p38. Based on these results, it has been proposed that neuronal differentiation of PC12 cells might require a sustained activation of either ERK or p38 MAPKs, combined with the transient activation of the other MAPK⁸⁵.

5) Osteogenic differentiation. p38 plays an important role in osteoblast differentiation in several cell systems. p38 and ERK are activated as calvarial osteoblast differentiate into mature osteocytes. When p38 was inhibited with pyridinil imidazoles, the expression of differentiation markers, such as alkaline phosphatase (ALP) and mineral deposition, were significantly reduced. MC3T3-E1 osteoblastic cells expressing dominant negative p38 also displayed a delayed mineral deposition and reduced ALP activity. Finally, differentiation of bone marrow osteoprecursors was also impeded by the p38 MAPK inhibitor, as justified by the same markers of osteogenic differentiation⁸⁶.

6) Chondrogenic differentiation. ATDC5 cells are differentiated by Growth/Differentiation factor-5 (GDF-5) that induces a potent activation of p38 and ERK. Phosphorylation of p38 was also induced by BMP-2 and TGF- β 1. An inhibitor of p38 α and p38 β , SB202190, caused complete inhibition of cartilage nodule formation induced by GDF-5, but failed to affect ALP activity. Expression of the type II collagen, a hallmark of chondrogenesis, was also induced by GDF-5 and strongly suppressed by SB202190. On the other hand, although the inhibitor of ERK, PD98059, blocked the phosphorylation of ERK by GDF-5, it inhibited neither ALP activity nor cartilage nodule formation⁸⁷. Treatment of chicken mesenchymal cells with cytochalasin D disrupted the actin cytoskeleton with concomitant chondrogenic differentiation⁸⁸. The chondrogenic process was accompanied by an increase in p38 activity and inhibition of p38 with SB203580 blocked this process⁸⁸.

7) Erythroid differentiation. Erythroid differentiation of K562 cells induced by cyclosporin A⁸⁹, and of SKT6 cells induced by erythropoietin (Epo)⁹⁰ is mediated by p38. ERK1-2, p38 and JNK-2 were rapidly and transiently activated by Epo. However, only SB203580 but not PD98059 could suppress Epo-induced differentiation. SB203580 also prevented cyclosporinA-induced

haemoglobin synthesis in K562 cells, while another MEK inhibitor U0126, enhanced it, suggesting that activation of p38 and inactivation of ERK are involved in erythroid differentiation⁸⁹.

p38 IN PROLIFERATION

When referring to the regulation of proliferative processes, the general rule would be that there is no general rule, as p38 exhibits the same phenomenology previously described for differentiation and apoptosis. It can exert either a positive or a negative control depending on the cell type and the mitogenic stimulus in question. There is now ample evidence to assert that p38 activation favours proliferation in several cellular systems.

Using specific inhibitors for MEK and p38, it was demonstrated that both ERK and p38 are critically involved in the transduction of a proliferative signal and cooperate in G-CSF-induced cell proliferation in hemopoietic cells⁹³. Factors that promote T cell proliferation IL-2 and IL-7, activate both JNK and p38. Inhibition of p38 with pyridinyl imidazoles correlated with suppression of IL-2/7-driven T cell proliferation⁹⁴. In both cases, the proliferative effect of p38 activation was mediated by its substrate MAPKAP-K2. There is also evidence that p38 can be activated by other hemopoietic growth factors, including Steel locus factor, colony stimulating factor-1, granulocyte/macrophage-colony stimulating factor, interferons and interleukin-3⁹⁵.

In Swiss 3T3 fibroblasts and PC12 cells, FGF-2 activates ERK with similar kinetics. By contrast, in PC12 cells p38 is activated weakly and transiently, while a much stronger and sustained activation of p38 is seen in FGF-2-treated fibroblasts. Inhibition of ERK blocks differentiation but has little effect on proliferation. Contrarily, inhibitors of p38 block proliferation but have no effect on differentiation⁹⁶.

On the other hand, and to complicate things further, p38 can behave as an antagonist of mitogenic signalling. This is best exemplified by the relationship among p38 and the components of the Ras/ERK pathway, reminiscent of the situations observed in the regulation of apoptosis though, quite expectedly, with opposed effects. In some cells like NIH3T3, p38 can be activated by Ras and this activation provides a negative feedback for Ras proliferative signalling⁹⁷. In agreement, in primary human fibroblasts Ras induces premature senescence through sequential activation of the MEK-ERK pathway and the MKK3/6-p38 pathway⁹⁸. Also, ectopic expression of MEKK3 induces G1 arrest and reverses Ras-mediated transformation in NIH3T3 fibroblasts by downregulating cyclin D1⁹⁹. Recent reports go a bit further, suggesting that down-regulation of p38 is an essential requirement for Ras-induced transformation¹⁰⁰.

Interactions between Ras/ERK and p38 MAPKs become even more complex when the different p38 isoforms are taken into account. A new twist has been recently provided by the direct interaction between p38 and ERK MAPKs as a mechanism for regulating ERKs functions. p38 can bind to ERK and this interaction downregulates ERK phosphorylation state¹⁰¹. Interestingly, Mxi2, a p38 α splice isoform with a striking sensitivity to growth factor stimulation²⁵ can also bind to ERK1/2, but as opposed to p38, upregulates its nuclear activity by sustaining its phosphorylation levels in the nucleus but not in the cytoplasm¹⁰². Since Mxi2 and p38 are splice variants of the same gene, this opens an attractive scenario in which, by switching the splicing taking place at the p38 locus, the signalling machinery could be programmed to up-or down-regulate ERK activity, with its subsequent alterations on the proliferative/survival signals resulting from ERK activation.

p38 IN THE REGULATION OF CELL CYCLE PROGRESSION

At this stage, it should come as no surprise that proteins with such profound effects on proliferation, differentiation and apoptosis should impinge significantly on the machinery that regulates cell cycle progression. The first evidences were obtained in CCL39 cells and in NIH3T3 fibroblasts in which p38 negatively regulated cell cycle progression in the G1/S transition, mediated by the inhibition of cyclin D1 expression. In cells expressing high levels of MKK3, a significant inhibition of mitogen-induced cyclin D1 expression was observed. Accordingly, inhibition of p38 with SB203580 augmented cyclin D1 protein levels¹⁰³. Cyclin D1 seems to be one of the favoured targets for p38-mediated control but other cell cycle checkpoints are also subject to p38 regulation.

Regarding the G1/S transition, the Rho GTPase Cdc42 can inhibit cell cycle progression at G1/S through a mechanism requiring the activation of p38¹⁰⁴. Likewise, a constitutively active MKK6 is also capable of inducing cell cycle arrest on G1 and senescence in a p38-dependent fashion¹⁰⁵. The growth-inhibitory effects of TGF- β are at least partially mediated by the TAK1-MKK6-p38K pathway. In renal tubular cells promoter activities in cyclins D1 and A genes and cell cycle progression are negatively regulated by the TAK1-MKK6-p38 pathway and positively regulated by the MEK-ERK pathway¹⁰⁶.

The G2/M checkpoint is also under the influence of p38 regulation. Indeed, recent reports indicate that regulation by p38 of the phosphorylation of the phosphatase Cdc25B is a critical step at this checkpoint⁶⁵. Another isoform p38 γ , is required for gamma-irradiation-induced G2 arrest. Activation of the MKK6-p38 γ cascade is sufficient to produce a halt at

the G2/M transition, while the expression of dominant negative MKK6 or p38 γ allows cells to escape the DNA damage-induced G2 arrest. p38 γ effects are mediated by Cds1/Chk2¹⁰⁷. In a similar fashion, in CCL39 and Rat-1 cells, MEKK3 induces cell cycle arrest at G2/M by a mechanism dependent on p38 α/β that involves the up-regulation of p21 and the inhibition of cyclinA/Cdk2 and cyclinB/Cdk1¹⁰⁸.

In the metaphase/anaphase or mitotic spindle check point p38, but not ERK or JNK, is activated when cells are arrested in M phase by the disruption of the spindle with nocodazole. Injection of activated p38 into cleaving *Xenopus* embryos induced mitotic arrest. Treatment of NIH 3T3 cells with a inhibitor of p38 compromised the spindle assembly checkpoint function, suggesting that p38 is essential in this process in somatic cell cycles¹⁰⁹. Persistent activation of p38 blocks foetal thymocyte development at the CD25(+)CD44(-) stage, but inactivation of p38 is required for further differentiation into CD4(+)CD8(+) thymocytes. The arrest in mitosis is partially responsible for the blockade of differentiation. Highlighting that p38 is a critical regulatory element of differentiation and proliferation during the early stages of thymocyte development¹¹⁰. There are also studies under physiological conditions that show how p38 is activated during normal mitosis in retinal neuroblasts. The cell cycle in the proliferative zone of the retina is tightly controlled and proceeds in synchrony with interkinetic migration of the neuroblast nuclei. Mitotic profiles in the developing retina are highly enriched in phosphorylated p38. Blockade of p38 activity with SB203580 transiently arrested neuroblasts at the metaphase-anaphase transition, while prolonged p38 inhibition induced cell death. p38 inhibition also induced an aberrant mitotic profile, with polarized chromosomes. Suggesting that p38 is required for proper cell cycle progression during the metaphase-anaphase transition¹¹¹.

INVASION AND METASTASIS

Once cellular transformation has taken place, a series of processes must ensue before full-blown cancer develops, namely: migration from the primary site and invasion of surrounding tissues. Intravasation and dissemination through the vascular system. Neocolonization at distant sites and secondary, metastatic growth, that in a similar fashion to the growth of the primary tumour, also requires neovascularization. p38 is also involved in most of these processes.

Migration of myocytes induced by PDGF can be blocked by p38 inhibitors, in a process mediated by HSP27 and F-actin polymerization¹¹². Likewise, increased migration of lung endothelial cells induced by Heregulin is p38-dependent, as SB203580 can readily inhibit the process¹¹³. Once again, a cross talk between

en p38 and ERK pathways seems determinant in the regulation of migration, as it is the ratio between the activities of these two kinases what dictates the migratory propensity of epithelial cells¹¹⁴.

Migration and subsequent invasion of surrounding tissue requires the destruction of the extracellular matrix and underlying basal laminas enclosing the primary growth. In these processes, the secretion of proteases cocktails from the tumour cells plays an essential role. A large body of evidence point to p38 as an important regulator of protease synthesis and secretion. In malignant melanoma cells a p38 inhibitor blocked the expression of matrix-metalloproteinase (MMP-2) and reduced the invasive potential of these cells¹¹⁵. In squamous cell carcinoma cell lines, phorbol ester-enhanced secretion of metalloproteinase 9 (MMP-9) and *in vitro* invasiveness, correlated with a strong activation of p38¹¹⁶. Expression of collagenases 3 (MMP-13) and 1 (MMP-1) in transformed keratinocytes are also p38-dependent (117). Apparently, the activation of p38 enhances MMP-1 and stromelysin (MMP-3) expression by stabilizing their mRNA¹¹⁸.

Once dissemination through the vascular system and extravasation have taken place, cancer cells may either resume growth, leading to overt metastasis, or enter a state of protracted dormancy. ERK/p38 activity ratio predicts whether the cells will proliferate or enter a state of quiescence. In breast, prostate, melanoma, and fibrosarcoma cell lines, the level of active ERK and the ERK/p38 activity ratio predict for this behaviour in approximately 90% of the cell lines tested. Modulation of ERK/p38 activity ratio by multiple pharmacological and genetic interventions confirms that low p38/ERK ratio promotes tumor growth, whereas a high p38/ERK ratio favours proliferative arrest¹¹⁹.

p38 MAPKs can also be pivotal elements in the growth of primary tumours and in the metastatic process by virtue of their role as regulators of vascularization. As a proof of it, p38 has been found to be activated in response to Vascular Endothelial Growth Factor (VEGF) in endothelial cells, in which p38 activity is also required to regulate cellular migration¹²⁰. Angiogenesis induced by TNF- α is partly mediated by ephrin A1. In endothelial cells, ephrin A1 induction can be blocked by pyridinyl imidazoles¹²¹. In a similar fashion, VEGF secretion is upregulated by IL-1 in human vascular smooth muscle cells by a mechanism involving p38¹²². Lastly, the Kaposi sarcoma-associated herpesvirus G protein-coupled receptor, a potent stimulator of neovascularization and angiogenesis, upregulates VEGF expression and secretion with the participation of ERK and p38 pathways¹²³.

CONCLUSIONS AND PERSPECTIVES

At this stage, it is undoubted that MAPKs play key role in the processes that bring about malignification.

Thus, modulating the activities of MAPKs as a means to prevent tumoral growth and progression is now a widespread anti neoplastic strategy at its initial stages. Indeed, it is clear that the activities of MAPKs, in particular p38, are subject to regulation upon exposure to diverse classes of anti tumoral agents. With increased evidence supporting a role for MAPK signalling in antitumour drug action, p38 regulators may have an enormous potential as chemotherapeutic drugs themselves or as chemosensitizing agents. However, it is becoming clear that these responses tend to be context-dependent, and can differ enormously depending on the cellular system and conditions. The biological outcomes of p38 activation/inhibition may be completely different depending on the cell type and subtle variations when interfering with the intensity and duration of p38 signals can result in profound, unexpected and sometimes unwanted effects. Therefore modulation of p38 as an anti tumoral strategy, although theoretically promising, must be at this stage regarded with extreme caution and must always be based on a broad knowledge of the biological idiosyncrasy of the type of tumour subject to treatment.

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