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Activation of mitogen-activated protein kinase pathways by the granulocyte colony-stimulating factor receptor: mechanisms and functional consequences

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1. ABSTRACT

The cytokine Granulocyte Colony Stimulating Factor (G-CSF) promotes proliferation, differentiation, survival and functional maturation of cells within the neutrophilic granulocyte lineage. G-CSF binds to its cell-surface receptor (G-CSFR) causing activation via homodimerisation and subsequent phosphorylation on four tyrosine residues of the receptor intracellular domain. This initiates a range of intracellular signalling events including the activation of Mitogen-Activated Protein Kinase (MAPK) pathways. G-CSF stimulates activation of the ERK 1/2 pathway, as well as the stress-activated JNK and p38 pathways, and the less-characterised ERK5/Big MAPK 1 pathway. Receptor mutagenesis studies have aided in the identification of regions of the G-CSFR that mediate specific activation of these MAPK pathways. In addition, the activation of individual MAPK pathways appears to contribute to distinct biological outcomes. Thus, MAPK activation may be an important mediator of the actions of G-CSF.

2. INTRODUCTION

When cells are exposed to environmental stimuli such as cytokines, growth factors or hormones, responses are initiated by intracellular signalling pathways. These pathways relay information to various intracellular compartments to initiate changes such as alterations in gene expression. One group of signal transduction pathways known as the Mitogen-Activated Protein Kinase (MAPK) pathways are activated in response to a variety of extracellular stimuli. Several MAPK pathways have now been identified, including the extracellular signal-regulated kinase (ERK) pathway, the c-Jun N-terminal kinase (JNK) pathway, the p38 pathway and the ERK5 (Big MAPK 1) pathway (1,2,3). This review focuses on the activation of these MAPK pathways by the cytokine Granulocyte Colony Stimulating Factor (G-CSF), describing our current understanding of the mechanisms and functional consequences of MAPK activation in cells following their exposure to G-CSF.

Table 1. Mitogen-Activated Protein Kinase Pathways activated in response to G-CSF

MAPK Pathway	Cell Type	G-CSFR region	Role	Inhibitor	Reference
ERK	BaF3	646 – 739 686 – 785	proliferation	- - PD98059	21, 30, 32, 82 31
	myeloid	Y764 membrane-proximal + Box 2 ?	proliferation	U0126 U0126 PD98059	83,84 86 87
	32D	?	differentiation	U0126	98
JNK	BaF3	Y763 Y763 686 – 715	? proliferation ?	- Tat-TIJIP -	32 149 30
p38	BaF3	626 – 726 and Y763 686 – 715	proliferation	SB203580	31 30
	OCT-AML1	?	proliferation	SB203580	189
	Stromal Endothelial	?	VCAM-1 expression	SB203580	191,192
ERK5	BaF3	686 – 715 and C-terminal	proliferation, survival	-	30

Stimulation with G-CSF results in activation of several MAPK pathways, including the ERK, JNK, p38 and ERK5 pathways. Studies have utilised approaches such as G-CSF receptor mutagenesis and MAPK pathway inhibitors to delineate the regions of the G-CSFR which mediate activation, and the biological consequences of MAPK activation.

3. GRANULOCYTE COLONY-STIMULATING FACTOR AND ITS RECEPTOR ACTIVATION

The critical biological activities of G-CSF have been described over the last 25 years (4). G-CSF is the major cytokine that promotes the proliferation, maturation, and survival of cells of the neutrophilic granulocyte lineage (5,6,7). In addition, G-CSF facilitates the functional activation of mature neutrophils by enhancing cytotoxic activity and chemotaxis (8,9). The selective knockout of the gene for *G-CSF* in mice causes chronic neutropenia and impairs the response to bacterial infection. The administration of G-CSF restores these defects. G-CSF is therefore essential for maintaining normal neutrophil production during "steady-state" granulopoiesis *in vivo* and contributes to "emergency" granulopoiesis during infections (7).

These biological effects of G-CSF are mediated via a specific high-affinity receptor, G-CSFR (10,11). The G-CSFR is expressed on cells from the myeloblast to the mature neutrophil, but not on any lymphoid, erythroid or megakaryocytic lineage cells (12). Thus, the selective action of G-CSF has been attributed to this relatively restricted expression.

The G-CSFR is a transmembrane glycoprotein with an apparent molecular mass of 130 to 150 kDa, and belongs to the class I family of haemopoietic cytokine receptors (13,14). Its single polypeptide chain comprises a large extracellular ligand-binding domain, a single membrane-spanning domain and a small intracellular domain. This intracellular domain of 187 amino acids lacks intrinsic tyrosine kinase activity but is considered as the signal transduction domain of the receptor. Of importance for G-CSFR signal transduction are conserved regions called Boxes 1, 2 and 3 as well as four tyrosine (Y) residues. In the murine G-CSFR, these tyrosines are numbered Y703, Y728, Y743 and Y763, while the numbering for the human G-CSFR is Y704, Y729, Y744 and Y764 (15,16,17).

In the earliest stages of signalling by G-CSF, the G-CSFR homodimerises and its four intracellular tyrosine residues are phosphorylated by non-receptor tyrosine kinases including Jak1, Jak2, Tyk2, Tec, Lyn, Syk and Hck (18-25). Of these, Jak1 and Lyn appear to be constitutively associated with the membrane-proximal region of the G-CSFR, while Syk, Hck and Jak2 appear to be recruited following G-CSF stimulation (24,19,21,25). Studies utilising cell lines deficient in these individual tyrosine kinases have shown they have distinct roles in G-CSF-stimulated events. Jak1, but not Jak2 or Tyk2, is essential for receptor tyrosine phosphorylation and STAT activation following G-CSF-stimulation (22), while Lyn, but not Syk, is required for proliferation (26,27). In addition, the profile of kinases activated by G-CSF in differentiating cell types differs from those activated in mature neutrophils (24,28,29). Thus, different tyrosine kinases are activated following G-CSF-stimulation depending on the stage of cellular maturation and/or the particular biological response invoked.

The events directly following tyrosine kinase activation are also complex, but specific intracellular signalling pathways appear to contribute to the distinct target cell responses. An evaluation of these subsequent signalling events revealed activation of the Jak-STAT pathway, the Phosphatidyl inositol 3'Kinase (PI3K)-Akt pathway, and multiple Mitogen-Activated Protein kinase (MAPK) pathways (30-34). Mutagenesis of the G-CSFR itself has been useful in delineating the regions of the G-CSFR required for mediating the activation of individual MAPK signalling pathways (35,36). An overview of these findings is presented in Table 1. The MAPK pathways are the subject of this review, and are considered in more detail in the following sections.

4. AN INTRODUCTION TO MITOGEN-ACTIVATED PROTEIN KINASE PATHWAYS

The mammalian MAPK pathways have been defined by the presence of a three-tiered protein kinase module, whereby a MAPK kinase kinase (MAPKKK) phosphorylates and activates a MAPK kinase (MAPKK), which in turn phosphorylates and activates a MAPK (37) (Table 2). The MAPK pathways are evolutionarily

Table 2. Intracellular events in MAPK pathways

MAPK Pathway	**ERK**	**JNK**		**p38**		**ERK5**	
Stimulus	Cytokines	Stresses	Cytokines	Stresses	Cytokines	Stresses	Cytokines, hormones
Receptor	Cytokine Receptors	?	Cytokine Receptors	?	Cytokine Receptors		GCSF-R, EGF-R, NGF-R, BDNF-R, GPCRs
Adaptor/enzyme	Shc, SHP-2 Grb2, Src	Nck, Dbl, Crk, Cas Shc, Grb2. TRAF6		Grb2, TRAF6 MyD88, IRAK		Src	
Exchange factor	Sos						
Small G-protein	Ras	Rac, cdc42, Rap, Ras		Ras, Rac, cdc42, Rap		Ras	
MAPKKKK		PAK, NIK, GLK, HPK, MST2, GCK		PAK, GCK			
MAPKKK	Raf-1, B-Raf, A-Raf	MEKK1, MEKK2, MEKK4, MLK2, MLK3, Tpl2, ASK, DLK, Tak1		MEKK1, MEKK2, MEKK3, MEKK4, MLK2, MLK3, ASK1, DLK, Tpl2, Tak1		MEKK2, MEKK3, Tpl2	
MAPKK	MEK1, MEK2	MKK4, MKK7		MKK3, MKK4, MKK6		MEK5	
MAPK	**ERK1, ERK2**	**JNK1, JNK2, JNK3**		**p38α, p38β, p38γ, p38δ**		**ERK5**	
Substrates	Elk-1, $p90^{RSK}$, Mnk, JunD	c-Jun, JunD, ATF2, Elk-1, p66ShcA, NF-Atc1, HSF-1, TAT3, Bcl. Itch, 14-3-3		MAPKAPK2, MNK1, PRAK, Tau, ATF1, ATF2, MEF2A, NFAT, Sap-2, Elk-1, Ets-1, p53		MEF2. Sap1a, CREB, c-Myc	

The MAPK pathways are activated by stimuli, and are composed of a series of intracellular steps, which result in phosphorylation of protein substrates. The general organisation of a MAPK pathway is shown in (a). Some of the known stimuli, cellular receptors, signalling intermediates implicated in each level of the pathway, and substrates are summarised for the ERK 1/2 (b), JNK (c), p38 (d), and ERK5 (e) pathways.

conserved across eukaryotes and appear to be involved in critical cellular processes such as development, proliferation, differentiation, apoptosis and survival (38,39).

Several distinct MAPK pathways have been identified (reviewed in (3)). The Extracellular signal-Regulated Kinase (ERK), c-Jun N-terminal Kinase (JNK) and p38 pathways remain the best characterised, however there are other MAPKs including ERK3, ERK5, ERK7 and ERK8 (3). The ERK, JNK and p38 pathways show a number of distinctive features including their activation in response to different stimuli, their different upstream regulatory proteins, and differences in substrates and biological outcomes (40,41). Therefore, despite the overall similarities in pathway organisation, the different MAPKs are likely to play distinct, non-redundant roles. Interestingly, the tyrosine phosphorylation of cytokine receptors such as the G-CSFR initiates the activation of multiple MAPK pathways. In the following sections, we consider the ERK, JNK, p38 and ERK5 pathways in greater detail and overview their roles in signalling downstream of the G-CSFR.

5. THE EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK) MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY

5.1. Properties, stimuli and functions of the ERK 1/2 pathway

ERK 1 and 2 were originally identified as two serine/threonine kinases of 44 and 42kDa, respectively, activated in response to insulin stimulation (1). The ERKs have since been shown to be activated by a wide range of growth promoting stimuli including cytokines, serum, oncogenes and tumour-promoting phorbol esters (42-47), as well as a range of cellular stresses (48-51).

It is now possible to sequentially describe the intracellular events leading to ERK1/2 activation (Table 2). One of the best-studied mechanisms requires the recruitment of the adaptor protein Shc via its phosphotyrosine-binding (PTB) domain to phosphorylated tyrosine residues of cytokine or growth factor receptors (52-56). The ensuing tyrosine phosphorylation of Shc allows its subsequent recruitment of the adaptor protein Grb2 via its Src Homology 2 (SH2) domain (57). The Src Homology 3 (SH3) domain of Grb2 is bound by the exchange factor Sos and this complex allows the relocalisation of Sos from the cytoplasm to the membrane (58). This brings Sos into proximity with its substrate, the small G-protein Ras, which is activated through the exchange of GDP for GTP (59,60).

Active Ras then initiates signaling via the three-tier kinase cascade in the cytosol. Thus, Ras-GTP recruits and activates the MAPKKK Raf, which phosphorylates the MAPKKs MEK1 and/or MEK2 on two serine residues (61). The activated MEKs are dual-specificity kinases that phosphorylate ERK 1 /2 on both the threonine and tyrosine residues of the Thr-Glu-Tyr motif within their activation loop sequences (62). The active ERKs can then phosphorylate substrates in the cytosol, such as $p90^{Rsk}$, or translocate to the nucleus and phosphorylate transcription factors such as Elk-1 (63-65). To date, more than 50 substrates for ERK1 or 2 have been identified (66). This emphasises the diversity of actions that ERKs are likely to play in intracellular signalling events.

The biological effects of ERK 1/2 activation include enhanced proliferation or oncogenic transformation, the suppression of apoptosis, and enhanced differentiation. In the haemopoietic system, the ERKs are critical in the processes regulated by multiple cytokines involved in normal hemopoiesis (67). A number of upstream activators of ERK are also known to be potent oncogenes. For example, the overexpression of constitutively active MEK1 transformed fibroblasts, whereas transformation by Ras or v-Src oncogenes could be reverted by a dominant negative MEK1 (68).

Furthermore, Raf has been identified as an oncogene, and its constitutive activation promotes transformation (69). Activating Ras mutations have a frequency of around 30% in myeloid leukaemia (70), although the Ras-Raf-ERK pathway is not the only Ras-induced pathway involved in cellular transformation (71). Thus, activation of the ERK 1/2 pathway is common in human neoplasia, both in solid tumors (72-75) and in haemopoietic malignancies (76,77).

The targeted disruption of the ERK1 and ERK2 genes in mice has revealed some redundant but also some specific, non-redundant functions. ERK1 (p44) knockout mice are viable and fertile, but exhibit defects in thymocyte proliferation and maturation, suggesting a specific role in thymocyte development (78). In contrast, knockout of ERK2 (p42) is embryonic lethal between days 6.5 and 11.5, due to a lack of mesoderm differentiation and increased apoptosis, and the prevention of placental angiogenesis (79-81). This supports a role for ERK 1/2 in developmental processes involving proliferation, survival and differentiation *in vivo* in a number of systems including the haemopoietic compartment.

5.2. Activation of the ERK 1/2 Pathway in Response to G-CSF

G-CSF stimulation activates the ERK 1/2 pathway in G-CSFR-expressing cells such as myeloid cell types, or in cells transfected to stably express the G-CSFR, such as the proB cell line BaF3 (21,31,82-87). Therefore, ERK activation is not merely a consequence of activation of overexpressed G-CSFRs. The functional consequences of ERK activation have been investigated in the BaF3 cell system. These cells are normally dependent on IL-3 for both proliferation and survival (88), but following their transfection with the G-CSFR they also proliferated in response to G-CSF (89). The presence of G-CSF promoted survival in the absence of IL-3, whilst its withdrawal initiated apoptotic cell death (90). This highlights that important biological events follow G-CSFR activation.

Early studies utilised the BaF3 cell system to identify the regions of the G-CSFR mediating ERK activation. Mutant forms of the G-CSFR were expressed and signalling events compared to those initiated by the wild-type receptor. Initial studies showed that truncation of the G-CSFR at residue 646, but not 739, abolished ERK activation as well as cell proliferation. This suggested the requirement for the membrane proximal 57 amino acids for signaling to the ERK 1/2 pathway (82,21). This analysis has been further refined utilising individual point mutations of the intracellular tyrosine residues (i.e. $\Psi \rightarrow$ F mutants of the G-CSFR). Interestingly and perhaps unexpectedly, this analysis revealed that ERK activation in BaF3 cells did not require any specific intracellular tyrosine residues. However, Ras activation was required and this was mediated by the membrane-proximal region (32). Studies in the myeloid cell line LGM-1 also revealed a role for the membrane-proximal region including Box 2 of the G-CSFR in mediating Jak2 and ERK activation (86). In contrast, in primary myeloid cells, ERK activation appeared to be mediated, at least partly, by Y764 (83,84), as both a Y764F and Y-Null G-CSFR failed to stimulate ERK 1/2 phosphorylation (83). Therefore, multiple regions of the G-CSFR signal transducing domain appear to be involved in ERK 1/2 activation, and there may be cell-type-specific differences in the regions required for this activation.

G-CSFR mutagenesis studies have also provided some information on the possible roles played by ERK activation. In BaF3 cells, G-CSFR mutagenesis indicated ERK 1/2 activation correlated with proliferation (21,31,82). However, this analysis has been refined with the more recent availability of specific protein kinase inhibitors. The judicious use of these inhibitors has allowed the role of post-receptor signalling events to be investigated. PD 98059 is a flavone compound that prevents the activation of ERK (91,92). Its use has confirmed the role of ERK 1/2 in BaF3 cell proliferative responses (31), and further implicated ERK 1/2 in proliferation signalling in both myeloid cell lines and primary cells (86,84).

The modulation of ERK 1/2 activation may be an important mechanism by which cytokines, such as G-CSF, control the balance between proliferation and differentiation. A study utilising the human myeloid MPD cell line as a model of G-CSF-induced neutrophil progenitor growth and maturation showed that treatment with PD 98059 alone decreased proliferation, while stimulation with G-CSF in the presence of inhibitor increased neutrophilic differentiation (87). Conversely, over-expression of a constitutively-active MEK mutant increased cell proliferation at the expense of differentiation (87). This is consistent with a role for G-CSF-stimulated ERK 1/2 activation in proliferation. It further suggests that in the absence of ERK 1/2 activation, G-CSF signalling directs neutrophilic differentiation.

In evaluating this balance between neutrophil proliferation and differentiation, a study in MPD neutrophil progenitors was conducted (85). While ERK 1/2 was activated in progenitors by both G-CSF and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), the ability for G-CSF to activate ERK 1/2 was lost both in differentiated MPD cells and isolated mature human neutrophils. In addition, G-CSF signalling to other signalling proteins such as STAT3 and Ras was retained. Interestingly, the broad phosphatase inhibitor pervanadate restored the ability of G-CSF to activate ERK 1/2, and the use of the SHP-1 specific inhibitor, sodium stibogluconate, implicated SHP-1 as a phosphatase involved in inhibiting ERK 1/2 activation (85). When these studies (87, 85) are considered together, a model may be proposed whereby G-CSF-mediated activation of ERK 1/2 is important for neutrophil progenitor proliferation, and the expansion of the immature granulocytic pool. G-CSF may initiate differentiation by activating SHP-1, which inhibits ERK 1/2 activation and proliferation, thus permitting terminal differentiation. This regulation of ERK 1/2 activation may allow optimal neutrophil production in response to G-CSF. Physiologically, this regulation may be exerted by co-stimulatory cytokines, such as GM-CSF.

Despite the attractiveness of this model, there are discrepancies in the conclusions drawn on the activation of ERK 1/2 by G-CSF and its subsequent roles. G-CSF did not activate ERK 1/2 in mature neutrophils isolated from the peripheral blood of human donors (85,87), whereas others have consistently shown G-CSF-stimulation of these cells resulting in ERK 1/2 phosphorylation (93-97). As both protocols isolate neutrophils from peripheral blood by dextran sedimentation followed by centrifugation in a Ficoll gradient, and hypotonic lysis of contaminating erythrocytes, the reasons for such consistent discrepancies remain unclear.

ERK dependent effects in G-CSF signalling have also been observed in isolated human neutrophils (94). Thus, G-CSF treatment of these mature neutrophils enhanced phosphorylation of ERK 1/2, actin depolymerisation and concomitant morphological changes, and impaired chemotaxis (94). Treatment with the MEK inhibitor, PD 98059, but not the p38 inhibitor, SB 203580, completely prevented actin rearrangement in response to G-CSF (94). This model suggests that G-CSF may utilise the ERK 1/2 pathway to modulate specific functions of mature neutrophils.

There are numerous studies in lymphoid and myeloid cell types which suggest a role for the ERK 1/2 pathway in G-CSF-stimulated proliferation. In addition, a role for ERK 1/2 in differentiation of 32D cells in response to G-CSF has been reported (98). Pre-treatment with a MEK1/2 inhibitor, U0126, reduced granulocytic differentiation of 32D cells 9 days after G-CSF-stimulation from 64.9% to 22.1%. Similarly, U0126 pre-treatment also potently inhibited granulocytic differentiation of the myeloid cell line HL-60 following stimulation with retinoic acid (31.8% reduced to 2.2%), suggesting the MEK/ERK 1/2 pathway regulates granulocytic differentiation of myeloid cells in response to distinct stimuli (98). Future studies assessing the effect of U0126 on the cytokine-stimulated differentiation of primary myeloid cells will be useful in evaluating the role of the MEK/ERK 1/2 pathway in granulocytic differentiation.

6. THE C-JUN N-TERMINAL KINASE (JNK) MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY

6.1. Properties, stimuli and functions of the JNK pathway

JNK was first described in 1990 as a 54kDa serine/threonine kinase activated following intraperitoneal injection of rats with the protein synthesis inhibitor cycloheximide (99). Two kinases of 46 and 55kDa were subsequently purified with the N-terminal activation domain of c-Jun, and thus named JNK 1 and 2 (100). Ten JNK isoforms have since been identified, which result from alternative splicing of three related genes, *JNK1*, *JNK2* and *JNK3* (101-103). *JNK1* and *JNK2* are ubiquitously expressed, while *JNK3* expression is restricted to the brain, heart and testes (104). The JNKs are primarily activated in response to stresses such as UV irradiation, heat shock, osmotic shock, oxidative stress and protein synthesis inhibitors, or the proinflammatory cytokines Tumour Necrosis Factor (TNF)-α and IL-1 (102,105-107). Haemopoietic cytokines such as G-CSF and IL-3 are also potent activators of the JNK pathway (32,108-111).

The JNK pathway displays similar organisation to the ERK 1/2 pathway. However, the JNK pathway is more complex, with numerous proteins potentially involved at each level (Table 2). As a consequence, the upstream events in the JNK pathway have remained poorly characterised (112,113). More than twelve different mammalian MAPKKKs activate the JNK pathway following their overexpression (40,114). The events leading to MAPKKK activation by stresses or cytokines, may involve various adaptor molecules (115,116), small G proteins (117) and Ste20 protein kinases (118,119). Activation of one or more MAPKKK leads to activation of MAPKKs. Two MAPKKs, MKK4 and MKK7, have been identified which directly phosphorylate both JNK1 and JNK2 on threonine and tyrosine residues of the Thr-Pro-Tyr motif within their activation loops (120,121). While there is evidence to suggest that MKK4 and MKK7 co-operate to achieve JNK activation (122), these kinases may also be differentially activated in response to cytokine or stress stimuli (123-125). Active JNK translocates to the nucleus where it phosphorylates various transcription factors including c-Jun, and activating transcription factor 2 (ATF2) (126-128). JNK also has a range of non-nuclear substrates including the Bcl family members (129), the E3 ligase Itch (130) and the 14-3-3 family of adaptor proteins (131) and thus is likely to mediate a variety of cellular responses.

The activation of JNK has been associated with diverse and often apparently contradictory cellular responses (132,133). These include proliferation, differentiation, survival, and apoptosis. Initially, the biological role of JNK was studied in various cell lines by overexpression of JNK, its upstream activators, and dominant negative kinases. More recently, the production of *JNK1*, *JNK2* and *JNK3* knockout mice has allowed more detailed study of JNK functions *in vitro* and *in vivo*.

JNK activation in response to cytokines results in varying biological outcomes in haemopoietic cells. Several studies suggest a role for JNK in proliferation or survival. The proliferation of FD-EPO cells following erythropoietin stimulation required JNK activation, while the transformation and survival of B lymphoblasts by the Bcr-Abl oncogene correlated with constitutive JNK activity (134-136). In addition, constitutive JNK activation is implicated in the progression of adult T-cell leukaemia following transformation by Human T cell Leukaemia virus type I (137). The treatment of FL5.12 lymphocytic cells with the quinone reductase inhibitor, dicumarol, suppressed JNK activation and survival following IGF-1 exposure, suggesting a link between JNK and survival (138). Similarly, thymocytes defective in JNK activation appeared more sensitive to FasL and TNFα-induced

apoptosis (139,140). Conversely, the activation of JNK and p38 following IL-7 withdrawal contributed to thymocyte apoptotic cell death (141). Thus it would appear that the biological effect of JNK activation is influenced by both the stimuli and cellular context.

The duration of JNK activation may also be important in determining cell fate. For example, treatment of Jurkat T-cells with phorbol 12-myristate 13-acetate (PMA) resulted in transient JNK activation and T-cell activation. However, γ-irradiation caused the persistent activation of JNK, but not ERK or p38, and induced apoptosis (142). Furthermore, the constitutive activation of JNK1 promoted apoptosis, while a dominant negative mutant of MEKK1 or JNK1 prevented cell death (142). Similarly, overexpression of wild-type or constitutively active ASK1, a MAPKKK upstream of JNK and p38, resulted in sustained activation of overexpressed JNK and p38 following oxidative stress, and induced apoptosis through mitochondrial-dependent caspase activation (143-145). Thus, transient JNK activation, like that occurring following cytokine stimulation, may promote cellular proliferation and survival. Sustained activation may result in the induction of apoptosis. The mediators of these different cellular outcomes remain to be identified.

When the *JNK* genes were individually disrupted, mice were viable, but had defects in immune responses and apoptosis. Both *JNK1* and *JNK2* appeared to regulate proliferation, maturation and survival in T lymphocytes (146,147). In contrast, the combined disruption of *JNK1* and *JNK2* genes caused early embryonic lethality, and a severe dysregulation of apoptosis in specific areas of the brain (148). Thus JNK appears to be required for aspects of haemopoietic and neuronal development.

6.2. Activation of the JNK Pathway in Response to G-CSF

While the JNK pathway has generally been considered a stress-activated pathway, haemopoietic cytokines including G-CSF also activate JNK (32,108). The requirement of the individual tyrosines of the G-CSFR for JNK activation was assessed in the BaF3 model cell system (32). The replacement of the three membrane proximal tyrosines of the G-CSFR individually did not affect JNK activation. However, loss of the membrane distal tyrosine, Y763, completely abrogated the activation of JNK in BaF3 cells (32, 149). This indicated a requirement for Y763 G-CSFR in the initiation of the JNK pathway following G-CSF-stimulation. Subsequently, the SH2 domain-containing adaptor proteins Shc, Grb2 and 3BP2, were identified as potential binding partners for Y763 of the G-CSFR utilising peptide-binding assays (149). The mutation of the Y763 G-CSFR interaction motif selectively prevented the binding of the Shc and Grb2 SH2 domains, yet JNK activation by G-CSF was retained, suggesting the direct binding of Shc or Grb2 to Y763 was not required. Thus the activation of the JNK pathway by Y763 of the G-CSFR appears to be mediated via interaction with other signalling intermediates (149). The identification of 3BP2 as potentially interacting with Y763 G-CSFR is of interest, as it has not been implicated in cytokine receptor signal transduction or MAPK signalling pathways specifically to date. 3BP2 is a 60kDa protein widely expressed in haemopoietic cells, and contains an N-terminal Pleckstrin Homology (PH) domain, a central proline-rich region, and a C-terminal SH2 domain (150). 3BP2 also contains 2 serine and 3 tyrosine phosphorylation sites, which bind 14-3-3 chaperone proteins and the SH2 domain-containing proteins Lyn, Vav and Phospholipase C-γ, respectively (151-153). Thus, 3BP2 may potentially interact with a large number of partners in a phosphorylation-dependent manner. As such, 3BP2 could aid in the assembly of a protein complex at the plasma membrane that leads to JNK activation (149).

While several studies have reported the absolute requirement for Y763 of the G-CSFR for JNK activation (32,149), conflicting observations have been made utilising various G-CSFR truncation mutants (30). When JNK was immunoprecipitated from G-CSF-stimulated BaF3 cell lysates expressing a Δ715 truncated G-CSFR (thus containing only Y704), its activity as judged by substrate phosphorylation was retained. However, truncation of the G-CSFR at amino acid 685 reduced phosphorylation (30). This suggested that the membrane proximal region of residues 686 to 715, rather than Y763 G-CSFR, was required for JNK activity of the G-CSFR. Although different methods were used, both studies quantitated JNK activity by phosphorylation of known *in vitro* JNK substrates, and therefore should have made similar observations. Interestingly, the same region of 686 to 715 of the G-CSFR was also shown to be required for ERK2 and p38 activation (30). Thus, the specific region of the G-CSFR required for G-CSF-stimulated JNK activation remains to be completely defined.

The biological role of cytokine-stimulated JNK activation has been investigated in BaF3 cells using G-CSFR mutants in which the membrane distal tyrosine was replaced (Y763F) (32). The proliferation of the Y763F G-CSFR mutant in G-CSF was comparable to that of the wild-type receptor, suggesting that JNK activation was not required for proliferation (32). However, the comparison of a G-CSFR mutant containing no intracellular tyrosines, which failed to proliferate, with a mutant containing only Y763, revealed that Y763 of the G-CSFR did contribute to transduction of a proliferative signal in response to G-CSF (149). As Y763 mediated JNK activation, the potential involvement of JNK was investigated further utilising a specific JNK-directed inhibitor. A JNK peptide inhibitor based on the JNK Binding Domain (residues 153 - 163) of the scaffold JIP-1, termed 'truncated Inhibitor of JNK-based on JIP-1' (TI-JIP), selectively bound JNK and inhibited JNK activity towards substrates including c-Jun *in vitro* (154). The addition of a cell permeable derivative of TI-JIP prevented the remaining proliferation of Y763 Only G-CSFR-expressing BaF3 cells following culture in G-CSF (149). This suggested that JNK activation initiated by Y763 G-CSFR promoted proliferation (149). Another JNK inhibitor with a distinct mechanism of action would

be of use in confirming a role for JNK in G-CSF-stimulated proliferation. Whilst SP600125 has been increasingly used in a number of *in vitro* studies in non-haemopoietic systems, its specificity has been questioned (156,157) and it will remain to be seen whether other inhibitors such as AS601245 (158,159) will be more useful in such studies.

7. THE P38 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY

7.1. Properties, stimuli and functions of the p38 pathway

p38 was discovered in 1993 as a protein that was rapidly tyrosine phosphorylated in response to the endotoxin, lipopolysaccharide (LPS) (160). Its subsequent cloning in 1994 revealed a protein of 43kDa, containing a Thr-Gly-Tyr dual phosphorylation motif, sharing homology with the other MAPK family members and acting as a serine/threonine kinase (161). The p38 subfamily is now known to be composed of four isoforms (p38α, p38β, p38γ and p38δ) with p38α and p38β being ubiquitously expressed. Along with the JNKs, the p38s are also referred to as SAPKs, due to their activation in response to cellular stresses, such as hyperosmolarity, UV and γ-irradiation, heat shock and proinflammatory cytokines (161-163). The p38s may also be activated by cytokines including GM-CSF and IL-3 (164).

While JNKs and p38s become activated in response to similar types of cellular stresses, the activation cascades appear to be distinct (Table 2). The MAPKKKs, MLK3, ASK1, MEKK4 and TAK1, have been implicated in mediating the upstream activation of both the JNK and p38 pathways (165). However, the pathways diverge at the level of the MAPKKs, where MKK4 activates JNK, but not p38 *in vivo* (62), while MKK3 and MKK6 activate p38 and not JNK (166,167). Substrates of the p38s include the kinases MAPKAPK2, MNK1 and PRAK, and the transcription factors ATF2, ELK-1, p53 and NFAT (reviewed in (123)).

Targeted inactivation of p38α in mice results in embryonic death due to a placental defect (168-170), thus providing little insight into the function of this p38 in the adult. Studies using cell lines have suggested an important role for p38s in cellular processes including apoptosis, proliferation, differentiation, inflammation and tumour suppression (see (171) for review). In haemopoietic cells, p38 has been implicated in macrophage and neutrophil functional responses, such as granular exocytosis, chemotaxis adherence and apoptosis (172). p38 also positively regulates the biosynthesis of TNF-α and IL-1β by monocytes and macrophages (173,174). These proinflammatory cytokines are involved in the pathogenesis of rheumatoid arthritis, endotoxin-induced shock, inflammatory bowel disease and osteoporosis (175,176).

The increasing availability of small molecule inhibitors of the p38s have provided alternative means for evaluating the physiological roles of MAPK activation. The pyridinyl imidazole compound SB 203580 inhibits p38α MAPK activity *in vitro* through competition at the ATP-binding site (177). Pre-incubation with SB203580 prevented the activation of MAPKAP kinase-2 by arsenite and the endotoxin LPS, establishing MAPKAP kinase-2 as a physiological substrate for p38α (178). Pyridinyl imidazoles have been shown to inhibit LPS-stimulated IL-1 and TNF-α biosynthesis in human monocytes (160,179) and to have therapeutic activity in inflammatory disease models (180). The potential application of SB 203580 and related compounds as anti-inflammatory therapies has led to the more recent development of other p38 inhibitors. RWJ 67657 is an orally-active pyridinyl imidazole inhibitor of p38α and p38β, with improved potency and selectivity over SB 203580 *in vitro* (181). RWJ 67657 is currently in clinical trials, and has shown potential in preventing endotoxin-induced renal damage (182).

Diaryl urea compounds have also now been described as a new class of p38 inhibitors. BIRB796 has little structural similarity to the pyridinyl imidazoles, and inhibits all p38 isoforms, by stabilizing a kinase conformation that is incompatible with ATP binding (183,184). BIRB796 reduced both LPS-stimulated TNF production and arthritis severity in a mouse model (184). BIRB796 also displayed anti-inflammatory effects in a trial of human endotoxemia and entered phase IIb/III clinical trials for the treatment of rheumatoid arthritis (184-187). Many of the commercially available kinase inhibitors are active against more than one kinase (156,188), therefore there is the potential that the observed effects in *in vitro* cell assays may be due to inhibition of unrelated kinases. Thus, the availability of several p38 inhibitors with distinct mechanisms of action will be useful to further elucidate the physiological roles of the p38s.

7.2. Activation of p38 in response to G-CSF

The regions of the G-CSFR involved in activation of the p38 pathway in response to G-CSF have been investigated by receptor mutagenesis studies in BaF3 cells. Two distinct regions were involved in p38 activation. The membrane-proximal 100 amino acid portion was sufficient for low levels of p38 phosphorylation and MAPKAPK2 activation. However, a Y763F mutant G-CSFR showed a strong reduction in p38 and MAPKAPK2 activation following G-CSF-stimulation, showing this second region of the G-CSFR was required for maximal activation of these kinases (31). These same regions had previously been implicated in controlling low and high levels of Ras activation respectively (32). Thus, the inducible expression of a dominant negative Ras mutant protein, confirmed that p38 activation by G-CSF was dependent on Ras at least in BaF3 cells, while activation by the stress stimuli, anisomycin, was Ras-independent (31). However, reminiscent of the studies on G-CSF-mediated JNK activation in BaF3 cells (32,149), another study produced conflicting results (30). Dong and colleagues utilised G-CSFR truncation mutants to assess p38 activation in response to G-CSF. While p38 activation was retained in the Δ715 G-CSFR truncation mutant, truncation at residue 685 abolished activation, suggesting

the region spanning residues 686 to 715 was required (30). While Rausch and Marshall utilised phospho-p38 immunoblotting and measured MAPKAPK2 phosphorylation of a substrate to show activation of these kinases, Dong and colleagues assayed p38 phosphorylation of the substrate ATF2 (30,31). Both assay techniques appear robust, therefore the reason for the discrepancies remains unclear.

As mentioned in the preceding section, SB 203580 inhibits p38 MAP kinases (173,178). In BaF3 cells, inhibition of p38 by SB 203580 halved the G-CSF-stimulated DNA synthesis in cells expressing the full length G-CSFR. Inhibition of the MEK/ERK 1/2 pathway by PD 98059 reduced G-CSF-induced DNA synthesis by a similar proportion, while a combination of both inhibitors was additive, reducing G-CSF-induced DNA synthesis by 80-90% (31). This suggests that in BaF3 cells, the ERK and p38 pathways co-operate to promote proliferation in response to G-CSF. This observation was confirmed in a leukaemic progenitor cell line (189), suggesting co-activation of the ERK and p38 pathways may be a common mechanism by which G-CSF promotes cell growth.

Interestingly, the ability to activate p38 appears to be selectively lost in mature neutrophils by G-CSF, while other cytokines, such as GM-CSF, IL-1β and TNFα, stimulate p38 phosphorylation (94-97). Thus, the differential activation of MAPK pathways by the cytokines G-CSF, GM-CSF, IL-1β and TNFα, may partly explain the different effects of these cytokines on neutrophil function.

Perhaps somewhat surprisingly, p38 activation by G-CSF may have specific functions in some non-haemopoietic cell types. While specific for the neutrophilic granulocyte lineage, the G-CSFR has been found on cell types within tissues that interact with haemopoietic cells, such as stromal and endothelial cells (190). Bone marrow stromal cells are important structural components of the bone marrow microenvironment, while the vascular endothelium is an interface between circulating blood components and extravascular tissues. G-CSF-stimulation of a stromal cell line or endothelial cells cultured *in vitro* resulted in activation of p38, and promoted the increased expression of adhesion molecules such as vascular cell adhesion molecule (VCAM-1). This significantly increased the adherence of isolated $CD34^+$ progenitors to the stromal cell layer (191), and increased the recruitment of leukocytes to an endothelial cell monolayer (192). These effects were blocked by G-CSFR antibody, or SB 203580 (191,192). The expression of adhesion receptors, such as VCAM-1, on the surface of stromal and endothelial cells within the bone marrow, may regulate self-renewal, differentiation and release of haemopoietic progenitors into the blood stream. Thus G-CSF, by stimulating activation of the p38 pathway in non-haemopoietic cells, may enhance the homing events of progenitor cells in the bone marrow microenvironment. Conversely, the down-regulation of this MAPK pathway may be one mechanism by which G-CSF promotes the mobilisation of $CD34^+$ haemopoietic progenitors from the bone marrow to circulating blood.

8. THE ERK5 OR BIG MITOGEN-ACTIVATED PROTEIN KINASE 1 PATHWAY

8.1. Properties, stimuli and function of the ERK5 pathway

ERK5 or Big MAPK1 (BMK1), cloned in 1995 (193,194), was the fourth member of the MAPK family to be identified, and is currently one of the least studied. ERK5 shares homology with ERK 1/2, and contains a Thr-Glu-Tyr sequence as its dual phosphorylation site within its activation loop (193,194). However, with a molecular mass of approximately 100kDa, ERK5 has a large C-terminal tail distinguishing it from the other MAPKs (194). ERK5 may be activated by stresses such as hyperosmolarity and oxidative stress, in addition to the growth factors EGF, NGF and Brain-Derived Neurotrophic Factor, and agents that act via G-protein-coupled receptors (195-198). Some of the upstream components of the ERK5 pathway have been identified, and include the tyrosine kinase Src; small G-protein Ras; the MAPKKKs MEKK2, MEKK3 and Tpl2, and the MAPKKs MEK5 (199,195,200). Its downstream targets include transcription factors of the myocyte enhancer factor 2 family, Sap1a and CREB (200) (Table 2). The dual-specificity phosphatases CL100 and MKP3, which were previously thought to be specific for ERK 1/2, have now been implicated in ERK5 inactivation (195).

Activation of the ERK5 pathway has been associated with proliferation, cell survival and differentiation. The ablation of ERK5 gene expression in mice results in embryonic lethality at E9.5 - 11.5, due to defects in cardiac development and angiogenesis (201-203). This suggests ERK5 is required for cell survival and normal development of the vascular system during embryogenesis.

8.2. Activation of the ERK5 Pathway in response to G-CSF

Despite being expressed in haemopoietic tissues including the bone marrow and spleen, there is a scarcity of studies describing the activation and function of ERK5 in haemopoietic cells. However, a role in haemopoietic cells may be implied, as ERK5 activation is also stimulated by G-CSF (30). G-CSF-stimulation strongly activated both MEK5 and ERK5 in BaF3 cells over-expressing the G-CSFR. Treatment with the tyrosine kinase inhibitor genistein prevented ERK5 activation, while ERK5 activity was enhanced by the protein kinase C-specific inhibitor GF109293X. Receptor mutagenesis showed the C-terminal region of the G-CSFR was required for ERK5 activation, with residues 686 - 715 being indispensable, and the remaining C-terminal amino acids playing an important role. The over-expression of dominant-negative and constitutively-active MEK5 mutants implicated the ERK5 pathway in proliferation, and cell survival in reduced concentrations of G-CSF (30). Thus ERK5 activation may mediate multiple biological effects of G-CSF-stimulation. Lending support to this

hypothesis, the presence of ERK5 has been reported in human neutrophils (204). The activity of MEK5 and ERK5 was stimulated by the bacterial tripeptide, formyl methionyl-leucyl-phenylalanine (fMLP), which functionally activates mature neutrophil cytotoxic actions. Thus, in addition to ERK and p38, ERK5 may also regulate neutrophil chemotactic responses (204). It therefore remains to be determined if ERK5 is activated in response to G-CSF-stimulation of physiologically relevant cell types such as neutrophils.

9. PERSPECTIVES

G-CSF can stimulate the activation of the ERK1/2, JNK, p38 and ERK5 MAPK families. The intracellular regions of the G-CSFR mediating activation of the individual MAPK pathways have been investigated predominantly using mutagenesis studies in heterologous cell systems such as the BaF3 cell line. These studies have shown the membrane-proximal region of the G-CSFR is sufficient to mediate activation of the ERK, p38 and ERK5 pathways. In contrast, the membrane-distal tyrosine residue, Y763, contributes to maximal p38 activation, and appears to be required for JNK activation in response to G-CSF. However, the regions of the G-CSFR mediating activation have not been completely defined, as discrepancies can be found in the literature, even when studies have been carried out in identical cell systems. Therefore, further studies are required to identify both the events initiated by the G-CSFR, and the subsequent downstream signalling from the G-CSFR, which culminates in MAPK activation. The increasing availability of specific inhibitors for signalling intermediates should facilitate such studies.

The activation of the MAPK pathways following stimulation with G-CSF may influence the biological outcomes in target cells. The current evidence suggests that activation of the ERK 1/2 pathway in immature or non-myeloid cell types promotes proliferation, consistent with its reported role in many non-haematopoietic cell types. As cells mature along the neutrophil lineage, the regulation of ERK 1/2 activation may be important in balancing pro-proliferation versus pro-differentiation signalling. G-CSF may also utilise the ERK 1/2 pathway to modulate the function of mature neutrophils. While the JNK, p38 and ERK5 pathways have all been implicated in proliferative responses to G-CSF in a lymphoid model system over-expressing the G-CSFR, their role in myeloid cells remains to be examined. The activation of the p38 pathway by G-CSF in stromal and endothelial cells has the potential to indirectly influence outcomes in haemopoietic cells, by modulating adhesive interactions with the haemopoietic microenvironment. Thus activation of the various MAPK pathways in response to G-CSF-stimulation appears to have diverse roles. The specific outcome is likely to be influenced by a combination of factors including the duration of MAPK activation, specific cellular context and other regulatory influences within the cell. It is apparent that further studies utilising multiple experimental approaches, including the overexpression of wild-type or mutant kinases, targeted disruption of MAPK genes in mice, and the use of specific kinase inhibitors in physiologically relevant cell types such mature neutrophils, will be required before the contribution of the individual MAPK pathways to G-CSF-mediated biological responses can be fully elucidated.

10. REFERENCES

1. Boulton, T. G., S. H. Nye, D. J. Robbins, N. Y. Ip, E. Radziejewska, S. D. Morgenbesser, R. A. DePinho, N. Panayotatos, M. H. Cobb, and G. D. Yancopoulos: ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 65, 663 - 675 (1991)

2. Kyriakis, J. M., J. R. Woodgett, and J. Avruch: The stress-activated protein kinases. A novel ERK subfamily responsive to cellular stress and inflammatory cytokines. *Ann N Y Acad Sci* 766, 303 - 319 (1995)

3. Bogoyevitch, M. A. and N. W. Court: Counting on mitogen-activated protein kinases--ERKs 3, 4, 5, 6, 7 and 8. *Cell Signal* 16, 1345 - 1354 (2004)

4. Roberts, A. W.: G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors* 23, 33 - 41 (2005)

5. Burgess, A. W. and D. Metcalf: The nature and action of granulocyte-macrophage colony stimulating factors. *Blood* 56, 947 - 958 (1980)

6. Burgess, A. W. and D. Metcalf: Characterization of a serum factor stimulating the differentiation of myelomonocytic leukemic cells. *Int J Cancer* 26, 647 - 654 (1980)

7. Lieschke, G. J., D. Grail, G. Hodgson, D. Metcalf, E. Stanley, C. Cheers, K. J. Fowler, S. Basu, Y. F. Zhan, and A. R. Dunn: Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood* 84, 1737 - 1746 (1994)

8. Lopez, A. F., N. A. Nicola, A. W. Burgess, D. Metcalf, F. L. Battye, W. A. Sewell, and M. Vadas: Activation of granulocyte cytotoxic function by purified mouse colony-stimulating factors. *J Immunol* 131, 2983 - 2988 (1983)

9. Colgan, S. P., P. W. Gasper, M. A. Thrall, T. C. Boone, A. M. Blancquaert, and W. J. Bruyninckx: Neutrophil function in normal and Chediak-Higashi syndrome cats following administration of recombinant canine granulocyte colony-stimulating factor. *Exp Hematol* 20, 1229 - 1234 (1992)

10. Nicola, N. A. and D. Metcalf: Binding of the differentiation-inducer, granulocyte-colony-stimulating factor, to responsive but not unresponsive leukemic cell lines. *Proc Natl Acad Sci U S A* 81, 3765 - 3769 (1984)

11. Demetri, G. D. and J. D. Griffin: Granulocyte colony-stimulating factor and its receptor. *Blood* 78, 2791 - 2808 (1991)

12. Nicola, N. A. and D. Metcalf: Binding of 125I-labeled granulocyte colony-stimulating factor to normal murine hemopoietic cells. *J Cell Physiol* 124, 313 - 321 (1985)

13. Fukunaga, R., E. Ishizaka-Ikeda, and S. Nagata: Purification and characterization of the receptor for murine granulocyte colony-stimulating factor. *J Biol Chem* 265, 14008 - 14015 (1990)

14. Nicola, N. A. and L. Peterson: Identification of distinct receptors for two hemopoietic growth factors (granulocyte

colony-stimulating factor and multipotential colony-stimulating factor) by chemical cross-linking. *J Biol Chem* 261, 12384 - 12389 (1986)
15. Fukunaga, R., Y. Seto, S. Mizushima, and S. Nagata: Three different mRNAs encoding human granulocyte colony-stimulating factor receptor. *Proc Natl Acad Sci U S A* 87, 8702 - 8706 (1990)
16. Fukunaga, R., E. Ishizaka-Ikeda, Y. Seto, and S. Nagata: Expression cloning of a receptor for murine granulocyte colony-stimulating factor. *Cell* 61, 341 - 350 (1990)
17. Pan, C. X., R. Fukunaga, S. Yonehara, and S. Nagata: Unidirectional cross-phosphorylation between the granulocyte colony-stimulating factor and interleukin 3 receptors. *J Biol Chem* 268, 25818 - 25823 (1993)
18. Horan, T., J. Wen, L. Narhi, V. Parker, A. Garcia, T. Arakawa, and J. Philo: Dimerization of the extracellular domain of granuloycte-colony stimulating factor receptor by ligand binding: a monovalent ligand induces 2:2 complexes. *Biochemistry* 35, 4886 - 4896 (1996)
19. Nicholson, S. E., A. C. Oates, A. G. Harpur, A. Ziemiecki, A. F. Wilks, and J. E. Layton: Tyrosine kinase JAK1 is associated with the granulocyte-colony-stimulating factor receptor and both become tyrosine-phosphorylated after receptor activation. *Proc Natl Acad Sci U S A* 91, 2985 - 2988 (1994)
20. Yoshikawa, A., H. Murakami, and S. Nagata: Distinct signal transduction through the tyrosine-containing domains of the granulocyte colony-stimulating factor receptor. *EMBO J* 14, 5288 - 5296 (1995)
21. Nicholson, S. E., U. Novak, S. F. Ziegler, and J. E. Layton: Distinct regions of the granulocyte colony-stimulating factor receptor are required for tyrosine phosphorylation of the signaling molecules JAK2, Stat3, and p42, p44MAPK. *Blood* 86, 3698 - 3704 (1995)
22.Shimoda, K., J. Feng, H. Murakami, S. Nagata, D. Watling, N. C. Rogers, G. R. Stark, I. M. Kerr, and J. N. Ihle: Jak1 plays an essential role for receptor phosphorylation and Stat activation in response to granulocyte colony-stimulating factor. *Blood* 90, 597 - 604 (1997)
23. Matsuda, T., M. Takahashi-Tezuka, T. Fukada, Y. Okuyama, Y. Fujitani, S. Tsukada, H. Mano, H. Hirai, O. N. Witte, and T. Hirano: Association and activation of Btk and Tec tyrosine kinases by gp130, a signal transducer of the interleukin-6 family of cytokines. *Blood* 85, 627 - 633 (1995)
24. Corey, S. J., A. L. Burkhardt, J. B. Bolen, R. L. Geahlen, L. S. Tkatch, and D. J. Tweardy: Granulocyte colony-stimulating factor receptor signaling involves the formation of a three-component complex with Lyn and Syk protein-tyrosine kinases. *Proc Natl Acad Sci U S A* 91, 4683 - 4687 (1994)
25. Ward, A. C., J. L. Monkhouse, X. F. Csar, I. P. Touw, and P. A. Bello: The Src-like tyrosine kinase Hck is activated by granulocyte colony-stimulating factor (G-CSF) and docks to the activated G-CSF receptor. *Biochem Biophys Res Commun* 251, 117 - 123 (1998)
26. Corey, S. J., P. M. Dombrosky-Ferlan, S. Zuo, E. Krohn, A. D. Donnenberg, P. Zorich, G. Romero, M. Takata, and T. Kurosaki: Requirement of Src kinase Lyn for induction of DNA synthesis by granulocyte colony-stimulating factor. *J Biol Chem* 273, 3230 - 3235 (1998)
27. Grishin, A., S. Sinha, V. Roginskaya, M. J. Boyer, J. Gomez-Cambronero, S. Zuo, T. Kurosaki, G. Romero, and S. J. Corey: Involvement of Shc and Cbl-PI 3-kinase in Lyn-dependent proliferative signaling pathways for G-CSF. *Oncogene* 19, 97 - 105 (2000)
28. Avalos, B. R., J. M. Parker, D. A. Ware, M. G. Hunter, K. A. Sibert, and B. J. Druker: Dissociation of the Jak kinase pathway from G-CSF receptor signaling in neutrophils. *Exp Hematol* 25, 160 - 168 (1997)
29. Miyazato, A., Y. Yamashita, K. Hatake, Y. Miura, K. Ozawa, and H. Mano: Tec protein tyrosine kinase is involved in the signaling mechanism of granulocyte colony-stimulating factor receptor. *Cell Growth Differ* 7, 1135 - 1139 (1996)
30. Dong, F., J. S. Gutkind, and A. C. Larner: Granulocyte colony-stimulating factor induces ERK5 activation, which is differentially regulated by protein-tyrosine kinases and protein kinase C. Regulation of cell proliferation and survival. *J Biol Chem* 276, 10811 - 10816 (2001)
31. Rausch, O. and C. J. Marshall: Cooperation of p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways during granulocyte colony-stimulating factor-induced hemopoietic cell proliferation. *J Biol Chem* 274, 4096 - 4105 (1999)
32. Rausch, O. and C. J. Marshall: Tyrosine 763 of the murine granulocyte colony-stimulating factor receptor mediates Ras-dependent activation of the JNK/SAPK mitogen-activated protein kinase pathway. *Mol Cell Biol* 17, 1170 - 1179 (1997)
33. Dong, F. and A. C. Larner: Activation of Akt kinase by granulocyte colony-stimulating factor (G-CSF): evidence for the role of a tyrosine kinase activity distinct from the Janus kinases. *Blood* 95, 1656 - 1662 (2000)
34. Chakraborty, A., S. M. White, T. S. Schaefer, E. D. Ball, K. F. Dyer, and D. J. Tweardy: Granulocyte colony-stimulating factor activation of Stat3 alpha and Stat3 beta in immature normal and leukemic human myeloid cells. *Blood* 88, 2442 - 2449 (1996)
35. Chakraborty, A., K. F. Dyer, M. Cascio, T. A. Mietzner, and D. J. Tweardy: Identification of a novel Stat3 recruitment and activation motif within the granulocyte colony-stimulating factor receptor. *Blood* 93, 15 - 24 (1999)
36. Hunter, M. G. and B. R. Avalos: Granulocyte colony-stimulating factor receptor mutations in severe congenital neutropenia transforming to acute myelogenous leukemia confer resistance to apoptosis and enhance cell survival. *Blood* 95, 2132 - 2137 (2000)
37. Garrington, T. P. and G. L. Johnson: Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol* 11, 211 - 218 (1999)
38. Davis, R. J.: Signal transduction by the c-Jun N-terminal kinase. *Biochem Soc Symp* 64, 1 - 12 (1999)
39. Strniskova, M., M. Barancik, and T. Ravingerova: Mitogen-activated protein kinases and their role in regulation of cellular processes. *Gen Physiol Biophys* 21, 231 - 255 (2002)
40. Davis, R. J.: Signal transduction by the JNK group of MAP kinases. *Cell* 103, 239 - 252 (2000)

41. Johnson, G. L. and R. Lapadat: Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 298, 1911 - 1912 (2002)
42. Whitehurst, C. E., T. G. Boulton, M. H. Cobb, and T. D. Geppert: Extracellular signal-regulated kinases in T cells. Anti-CD3 and 4 beta-phorbol 12-myristate 13-acetate-induced phosphorylation and activation. *J Immunol* 148, 3230 - 3237 (1992)
43. Leevers, S. J. and C. J. Marshall: Activation of extracellular signal-regulated kinase, ERK2, by p21ras oncoprotein. *EMBO J* 11, 569 - 574 (1992)
44. Fairhurst, R. M., M. Daeipour, M. C. Amaral, and A. E. Nel: Activation of mitogen-activated protein kinase/ERK-2 in phytohaemagglutin in blasts by recombinant interleukin-2: contrasting features with CD3 activation. *Immunology* 79, 112 - 118 (1993)
45. Casillas, A. M., K. Amaral, S. Chegini-Farahani, and A. E. Nel: Okadaic acid activates p42 mitogen-activated protein kinase (MAP kinase; ERK-2) in B-lymphocytes but inhibits rather than augments cellular proliferation: contrast with phorbol 12-myristate 13-acetate. *Biochem J* 290 (Pt 2), 545 - 550 (1993)
46. Loeb, D. M., H. Tsao, M. H. Cobb, and L. A. Greene: NGF and other growth factors induce an association between ERK1 and the NGF receptor, gp140prototrk. *Neuron* 9, 1053 - 1065 (1992)
47. Troppmair, J., J. T. Bruder, H. Munoz, P. A. Lloyd, J. Kyriakis, P. Banerjee, J. Avruch, and U. R. Rapp: Mitogen-activated protein kinase/extracellular signal-regulated protein kinase activation by oncogenes, serum, and 12-O-tetradecanoylphorbol-13-acetate requires Raf and is necessary for transformation. *J Biol Chem* 269, 7030 - 7035 (1994)
48. Chiri, S., S. Bogliolo, J. Ehrenfeld, and B. Ciapa: Activation of extracellular signal-regulated kinase ERK after hypo-osmotic stress in renal epithelial A6 cells. *Biochim Biophys Acta* 1664, 224 - 229 (2004)
49. Schliess, F., R. Schreiber, and D. Haussinger: Activation of extracellular signal-regulated kinases Erk-1 and Erk-2 by cell swelling in H4IIE hepatoma cells. *Biochem J* 309 (Pt 1), 13 - 17 (1995)
50. Yang, X. Y., Z. Zhang, and D. M. Cohen: ERK activation by urea in the renal inner medullary mIMCD3 cell line. *Am J Physiol* 277, F176 - F185 (1999)
51. Schmid-Alliana, A., L. Menou, S. Manie, H. Schmid-Antomarchi, M. A. Millet, S. Giuriato, B. Ferrua, and B. Rossi: Microtubule integrity regulates src-like and extracellular signal-regulated kinase activities in human pro-monocytic cells. Importance for interleukin-1 production. *J Biol Chem* 273, 3394 - 3400 (1998)
52. Tarr, P. E., R. Roncarati, G. Pelicci, P. G. Pelicci, and L. D'Adamio: Tyrosine phosphorylation of the beta-amyloid precursor protein cytoplasmic tail promotes interaction with Shc. *J Biol Chem* 277, 16798 - 16804 (2002)
53. Sasaoka, T., M. Ishiki, T. Wada, H. Hori, H. Hirai, T. Haruta, H. Ishihara, and M. Kobayashi: Tyrosine phosphorylation-dependent and -independent role of Shc in the regulation of IGF-1-induced mitogenesis and glycogen synthesis. *Endocrinology* 142, 5226 - 5235 (2001)
54. Dunican, D. J., E. J. Williams, F. V. Howell, and P. Doherty: Selective inhibition of fibroblast growth factor (FGF)-stimulated mitogenesis by a FGF receptor-1-derived phosphopeptide. *Cell Growth Differ* 12, 255 - 264 (2001)
55. Hermanns, H. M., S. Radtke, F. Schaper, P. C. Heinrich, and I. Behrmann: Non-redundant signal transduction of interleukin-6-type cytokines. The adapter protein Shc is specifically recruited to rhe oncostatin M receptor. *J Biol Chem* 275, 40742 - 40748 (2000)
56. Sakaguchi, K., Y. Okabayashi, Y. Kido, S. Kimura, Y. Matsumura, K. Inushima, and M. Kasuga: Shc phosphotyrosine-binding domain dominantly interacts with epidermal growth factor receptors and mediates Ras activation in intact cells. *Mol Endocrinol* 12, 536 - 543 (1998)
57. van der Geer, P., S. Wiley, V. K. Lai, J. P. Olivier, G. D. Gish, R. Stephens, D. Kaplan, S. Shoelson, and T. Pawson: A conserved amino-terminal Shc domain binds to phosphotyrosine motifs in activated receptors and phosphopeptides. *Curr Biol* 5, 404 - 412 (1995)
58. Lowenstein, E. J., R. J. Daly, A. G. Batzer, W. Li, B. Margolis, R. Lammers, A. Ullrich, E. Y. Skolnik, D. Bar-Sagi, and J. Schlessinger: The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 70, 431 - 442 (1992)
59. Egan, S. E., B. W. Giddings, M. W. Brooks, L. Buday, A. M. Sizeland, and R. A. Weinberg: Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 363, 45 - 51 (1993)
60. Boriack-Sjodin, P. A., S. M. Margarit, D. Bar-Sagi, and J. Kuriyan: The structural basis of the activation of Ras by Sos. *Nature* 394, 337 - 343 (1998)
61. Kyriakis, J. M., H. App, X. F. Zhang, P. Banerjee, D. L. Brautigan, U. R. Rapp, and J. Avruch: Raf-1 activates MAP kinase-kinase. *Nature* 358, 417 - 421 (1992)
62. Derijard, B., J. Raingeaud, T. Barrett, I. H. Wu, J. Han, R. J. Ulevitch, and R. J. Davis: Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267, 682 - 685 (1995)
63. Gille, H., M. Kortenjann, O. Thomae, C. Moomaw, C. Slaughter, M. H. Cobb, and P. E. Shaw: ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation. *EMBO J* 14, 951 - 962 (1995)
64. Frodin, M. and S. Gammeltoft: Role and regulation of 90 kDa ribosomal S6 kinase (RSK) in signal transduction. *Mol Cell Endocrinol* 151, 65 - 77 (1999)
65. Khokhlatchev, A. V., B. Canagarajah, J. Wilsbacher, M. Robinson, M. Atkinson, E. Goldsmith, and M. H. Cobb: Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* 93, 605 - 615 (1998)
66. Pearson, G., F. Robinson, G. T. Beers, B. E. Xu, M. Karandikar, K. Berman, and M. H. Cobb: Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 22, 153 - 183 (2001)
67. Platanias, L. C.: Map kinase signaling pathways and hematologic malignancies. *Blood* 101, 4667 - 4679 (2003)
68. Cowley, S., H. Paterson, P. Kemp, and C. J. Marshall: Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. *Cell* 77, 841 - 852 (1994)
69. Smith, M. R., G. Heidecker, U. R. Rapp, and H. F. Kung: Induction of transformation and DNA synthesis after

microinjection of raf proteins. *Mol Cell Biol* 10, 3828 - 3833 (1990)
70. Bos, J. L.: Ras oncogenes in human cancer: a review. *Cancer Res* 49, 4682 -4689 (1989)
71. Hamad, N. M., J. H. Elconin, A. E. Karnoub, W. Bai, J. N. Rich, R. T. Abraham, C. J. Der, and C. M. Counter: Distinct requirements for Ras oncogenesis in human versus mouse cells. *Genes Dev* 16, 2045 - 2057 (2002)
72. Oka, H., Y. Chatani, R. Hoshino, O. Ogawa, Y. Kakehi, T. Terachi, Y. Okada, M. Kawaichi, M. Kohno, and O. Yoshida: Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. *Cancer Res* 55, 4182 - 4187 (1995)
73. Sivaraman, V. S., H. Wang, G. J. Nuovo, and C. C. Malbon: Hyperexpression of mitogen-activated protein kinase in human breast cancer. *J Clin Invest* 99, 1478 - 1483 (1997)
74. Hoshino, R., Y. Chatani, T. Yamori, T. Tsuruo, H. Oka, O. Yoshida, Y. Shimada, Ari-i S, H. Wada, J. Fujimoto, and M. Kohno: Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* 18, 813 - 822 (1999)
75. Davies, H., G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B. A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J. W. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Seigler, T. L. Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton, and P. A. Futreal: Mutations of the BRAF gene in human cancer. *Nature* 417, 949 - 954 (2002)
76. Towatari, M., H. Iida, M. Tanimoto, H. Iwata, M. Hamaguchi, and H. Saito: Constitutive activation of mitogen-activated protein kinase pathway in acute leukemia cells. *Leukemia* 11, 479 - 484 (1997)
77. Kim, S. C., J. S. Hahn, Y. H. Min, N. C. Yoo, Y. W. Ko, and W. J. Lee: Constitutive activation of extracellular signal-regulated kinase in human acute leukemias: combined role of activation of MEK, hyperexpression of extracellular signal-regulated kinase, and downregulation of a phosphatase, PAC1. *Blood* 93, 3893 - 3899 (1999)
78. Pages, G., S. Guerin, D. Grall, F. Bonino, A. Smith, F. Anjuere, P. Auberger, and J. Pouyssegur: Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. *Science* 286, 1374 - 1377 (1999)
79. Hatano, N., Y. Mori, M. Oh-hora, A. Kosugi, T. Fujikawa, N. Nakai, H. Niwa, J. Miyazaki, T. Hamaoka, and M. Ogata: Essential role for ERK2 mitogen-activated protein kinase in placental development. *Genes Cells* 8, 847 - 856 (2003)
80. Yao, Y., W. Li, J. Wu, U. A. Germann, M. S. Su, K. Kuida, and D. M. Boucher: Extracellular signal-regulated kinase 2 is necessary for mesoderm differentiation. *Proc Natl Acad Sci U S A* 100, 12759 - 12764 (2003)
81. Saba-El-Leil, M. K., F. D. Vella, B. Vernay, L. Voisin, L. Chen, N. Labrecque, S. L. Ang, and S. Meloche: An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development. *EMBO Rep* 4, 964 - 968 (2003)
82. Bashey, A., L. Healy, and C. J. Marshall: Proliferative but not nonproliferative responses to granulocyte colony-stimulating factor are associated with rapid activation of the p21ras/MAP kinase signalling pathway. *Blood* 83, 949 - 957 (1994)
83. Akbarzadeh, S., A. C. Ward, D. O. McPhee, W. S. Alexander, G. J. Lieschke, and J. E. Layton: Tyrosine residues of the granulocyte colony-stimulating factor receptor transmit proliferation and differentiation signals in murine bone marrow cells. *Blood* 99, 879 - 887 (2002)
84. Hermans, M. H., G. J. van de Geijn, C. Antonissen, J. Gits, D. van Leeuwen, A. C. Ward, and I. P. Touw: Signaling mechanisms coupled to tyrosines in the granulocyte colony-stimulating factor receptor orchestrate G-CSF-induced expansion of myeloid progenitor cells. *Blood* 101, 2584 - 2590 (2003)
85. Baumann, M., T. Frye, T. Naqvi, and J. Gomez-Cambronero: Normal neutrophil maturation is associated with selective loss of MAP kinase activation by G-CSF. *Leuk Res* 29, 73 - 78 (2005)
86. Koay, D. C., T. Nguyen, and A. C. Sartorelli: Distinct region of the granulocyte colony-stimulating factor receptor mediates proliferative signaling through activation of Janus kinase 2 and p44/42 mitogen-activated protein kinase. *Cell Signal* 14, 239 - 247 (2002)
87. Baumann, M. A., C. C. Paul, S. Lemley-Gillespie, M. Oyster, and J. Gomez-Cambronero: Modulation of MEK activity during G-CSF signaling alters proliferative versus differentiative balancing. *Am J Hematol* 68, 99 - 105 (2001)
88. Palacios, R. and M. Steinmetz: Il-3-dependent mouse clones that express B-220 surface antigen, contain Ig genes in germ-line configuration, and generate B lymphocytes *in vivo*. *Cell* 41, 727 - 734 (1985)
89. Metcalf, D., T. Willson, M. Rossner, and P. Lock: Receptor insertion into factor-dependent murine cell lines to develop specific bioassays for murine G-CSF and M-CSF and human GM-CSF. *Growth Factors* 11, 145 - 152 (1994)
90. Rodriguez-Tarduchy, G., M. Collins, and A. Lopez-Rivas: Regulation of apoptosis in interleukin-3-dependent hemopoietic cells by interleukin-3 and calcium ionophores. *EMBO J* 9, 2997 - 3002 (1990)
91. Alessi, D. R., A. Cuenda, P. Cohen, D. T. Dudley, and A. R. Saltiel: PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase *in vitro* and *in vivo*. *J Biol Chem* 270, 27489 - 27494 (1995)
92. Dudley, D. T., L. Pang, S. J. Decker, A. J. Bridges, and A. R. Saltiel: A synthetic inhibitor of the mitogen-activated protein kinase cascade. *Proc Natl Acad Sci U S A* 92, 7686 - 7689 (1995)
93. Kitagawa, S., F. Takaku, and S. Sakamoto: A comparison of the superoxide-releasing response in human polymorphonuclear leukocytes and monocytes. *J Immunol* 125, 359 - 364 (1980)
94. Kutsuna, H., K. Suzuki, N. Kamata, T. Kato, F. Hato, K. Mizuno, H. Kobayashi, M. Ishii, and S. Kitagawa: Actin reorganization and morphological changes in human neutrophils stimulated by TNF, GM-CSF, and G-CSF: the role of MAP kinases. *Am J Physiol Cell Physiol* 286, C55 - C64 (2004)

95. Suzuki, K., M. Hino, F. Hato, N. Tatsumi, and S. Kitagawa: Cytokine-specific activation of distinct mitogen-activated protein kinase subtype cascades in human neutrophils stimulated by granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor-alpha. *Blood* 93, 341 - 349 (1999)
96. Suzuki, K., T. Hasegawa, C. Sakamoto, Y. M. Zhou, F. Hato, M. Hino, N. Tatsumi, and S. Kitagawa: Cleavage of mitogen-activated protein kinases in human neutrophils undergoing apoptosis: role in decreased responsiveness to inflammatory cytokines. *J Immunol* 166, 1185 - 1192 (2001)
97. Suzuki, K., M. Hino, H. Kutsuna, F. Hato, C. Sakamoto, T. Takahashi, N. Tatsumi, and S. Kitagawa: Selective activation of p38 mitogen-activated protein kinase cascade in human neutrophils stimulated by IL-1beta. *J Immunol* 167, 5940 - 5947 (2001)
98. Miranda, M. B., T. F. McGuire, and D. E. Johnson: Importance of MEK-1/-2 signaling in monocytic and granulocytic differentiation of myeloid cell lines. *Leukemia* 16, 683 - 692 (2002)
99. Kyriakis, J. M. and J. Avruch: pp54 microtubule-associated protein 2 kinase. A novel serine/threonine protein kinase regulated by phosphorylation and stimulated by poly-L-lysine. *J Biol Chem* 265, 17355 - 17363 (1990)
100. Hibi, M., A. Lin, T. Smeal, A. Minden, and M. Karin: Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7, 2135 - 2148 (1993)
101. Gupta, S., T. Barrett, A. J. Whitmarsh, J. Cavanagh, H. K. Sluss, B. Derijard, and R. J. Davis: Selective interaction of JNK protein kinase isoforms with transcription factors. *EMBO J* 15, 2760 - 2770 (1996)
102. Derijard, B., M. Hibi, I. H. Wu, T. Barrett, B. Su, T. Deng, M. Karin, and R. J. Davis: JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76, 1025 - 1037 (1994)
103. Kallunki, T., B. Su, I. Tsigelny, H. K. Sluss, B. Derijard, G. Moore, R. Davis, and M. Karin: JNK2 contains a specificity-determining region responsible for efficient c-Jun binding and phosphorylation. *Genes Dev* 8, 2996 - 3007 (1994)
104. Yang, D. D., C. Y. Kuan, A. J. Whitmarsh, M. Rincon, T. S. Zheng, R. J. Davis, P. Rakic, and R. A. Flavell: Absence of excitotoxicity-induced apoptosis in the hippocampus of mice lacking the Jnk3 gene. *Nature* 389, 865 - 870 (1997)
105. Cano, E., C. A. Hazzalin, and L. C. Mahadevan: Anisomycin-activated protein kinases p45 and p55 but not mitogen-activated protein kinases ERK-1 and -2 are implicated in the induction of c-fos and c-jun. *Mol Cell Biol* 14, 7352 - 7362 (1994)
106. Kyriakis, J. M. and J. Avruch: Protein kinase cascades activated by stress and inflammatory cytokines. *Bioessays* 18, 567 - 577 (1996)
107. Sluss, H. K., T. Barrett, B. Derijard, and R. J. Davis: Signal transduction by tumor necrosis factor mediated by JNK protein kinases. *Mol Cell Biol* 14, 8376 - 8384 (1994)
108. Terada, K., Y. Kaziro, and T. Satoh: Ras-dependent activation of c-Jun N-terminal kinase/stress-activated protein kinase in response to interleukin-3 stimulation in hematopoietic BaF3 cells. *J Biol Chem* 272, 4544 - 4548 (1997)
109. Foltz, I. N. and J. W. Schrader: Activation of the stress-activated protein kinases by multiple hematopoietic growth factors with the exception of interleukin-4. *Blood* 89, 3092 - 3096 (1997)
110. Crawley, J. B., L. Rawlinson, F. V. Lali, T. H. Page, J. Saklatvala, and B. M. Foxwell: T cell proliferation in response to interleukins 2 and 7 requires p38MAP kinase activation. *J Biol Chem* 272, 15023 - 15027 (1997)
111. Nagata, Y., E. Nishida, and K. Todokoro: Activation of JNK signaling pathway by erythropoietin, thrombopoietin, and interleukin-3. *Blood* 89, 2664 - 2669 (1997)
112. Nihalani, D., D. Meyer, S. Pajni, and L. B. Holzman: Mixed lineage kinase-dependent JNK activation is governed by interactions of scaffold protein JIP with MAPK module components. *EMBO J* 20, 3447 - 3458 (2001)
113. Kim, I. J., K. W. Lee, B. Y. Park, J. K. Lee, J. Park, I. Y. Choi, S. J. Eom, T. S. Chang, M. J. Kim, Y. I. Yeom, S. K. Chang, Y. D. Lee, E. J. Choi, and P. L. Han: Molecular cloning of multiple splicing variants of JIP-1 preferentially expressed in brain. *J Neurochem* 72, 1335 - 1343 (1999)
114. Chen, W., M. A. White, and M. H. Cobb: Stimulus-specific requirements for MAP3 kinases in activating the JNK pathway. *J Biol Chem* 277, 49105 - 49110 (2002)
115. Pomerance, M., M. C. Multon, F. Parker, C. Venot, J. P. Blondeau, B. Tocque, and F. Schweighoffer: Grb2 interaction with MEK-kinase 1 is involved in regulation of Jun-kinase activities in response to epidermal growth factor. *J Biol Chem* 273, 24301 - 24304 (1998)
116. Hashimoto, A., M. Kurosaki, N. Gotoh, M. Shibuya, and T. Kurosaki: Shc regulates epidermal growth factor-induced activation of the JNK signaling pathway. *J Biol Chem* 274, 20139 - 20143 (1999)
117. Minden, A., A. Lin, F. X. Claret, A. Abo, and M. Karin: Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81, 1147 - 1157 (1995)
118. Kiefer, F., L. A. Tibbles, M. Anafi, A. Janssen, B. W. Zanke, N. Lassam, T. Pawson, J. R. Woodgett, and N. N. Iscove: HPK1, a hematopoietic protein kinase activating the SAPK/JNK pathway. *EMBO J* 15, 7013 - 7025 (1996)
119. Pombo, C. M., J. H. Kehrl, I. Sanchez, P. Katz, J. Avruch, L. I. Zon, J. R. Woodgett, T. Force, and J. M. Kyriakis: Activation of the SAPK pathway by the human STE20 homologue germinal centre kinase. *Nature* 377, 750 - 754 (1995)
120. Whitmarsh, A. J. and R. J. Davis: Transcription factor AP-1 regulation by mitogen-activated protein kinase signal transduction pathways. *J Mol Med* 74, 589 - 607 (1996)
121. Fleming, Y., C. G. Armstrong, N. Morrice, A. Paterson, M. Goedert, and P. Cohen: Synergistic activation of stress-activated protein kinase 1/c-Jun N-terminal kinase (SAPK1/JNK) isoforms by mitogen-activated protein kinase kinase 4 (MKK4) and MKK7. *Biochem J* 352 Pt 1, 145 - 154 (2000)
122. Wada, T., K. Nakagawa, T. Watanabe, G. Nishitai, J. Seo, H. Kishimoto, D. Kitagawa, T. Sasaki, J. M. Penninger, H. Nishina, and T. Katada: Impaired synergistic

activation of stress-activated protein kinase SAPK/JNK in mouse embryonic stem cells lacking SEK1/MKK4: different contribution of SEK2/MKK7 isoforms to the synergistic activation. *J Biol Chem* 276, 30892 - 30897 (2001)
123. Kyriakis, J. M. and J. Avruch: Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev* 81, 807 - 869 (2001)
124. Yang, D., C. Tournier, M. Wysk, H. T. Lu, J. Xu, R. J. Davis, and R. A. Flavell: Targeted disruption of the MKK4 gene causes embryonic death, inhibition of c-Jun NH2-terminal kinase activation, and defects in AP-1 transcriptional activity. *Proc Natl Acad Sci U S A* 94, 3004 - 3009 (1997)
125. Tournier, C., C. Dong, T. K. Turner, S. N. Jones, R. A. Flavell, and R. J. Davis: MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. *Genes Dev* 15, 1419 - 1426 (2001)
126. Minden, A. and M. Karin: The JNK family of MAP kinases: regulation and function. *Hormones and Signalling* 1, 209 - 233 (1998)
127. Ip, Y. T. and R. J. Davis: Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. *Curr Opin Cell Biol* 10, 205 - 219 (1998)
128. Pulverer, B. J., J. M. Kyriakis, J. Avruch, E. Nikolakaki, and J. R. Woodgett: Phosphorylation of c-jun mediated by MAP kinases. *Nature* 353, 670 - 674 (1991)
129. Lei, K. and R. J. Davis: JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci U S A* 100, 2432 - 2437 (2003)
130. Gao, M., T. Labuda, Y. Xia, E. Gallagher, D. Fang, Y. C. Liu, and M. Karin: Jun turnover is controlled through JNK-dependent phosphorylation of the E3 ligase Itch. *Science* 306, 271 - 275 (2004)
131. Sunayama, J., F. Tsuruta, N. Masuyama, and Y. Gotoh: JNK antagonizes Akt-mediated survival signals by phosphorylating 14-3-3. *J Cell Biol* 170, 295 - 304 (2005)
132. Dunn, C., C. Wiltshire, A. MacLaren, and D. A. Gillespie: Molecular mechanism and biological functions of c-Jun N-terminal kinase signalling via the c-Jun transcription factor. *Cell Signal* 14, 585 - 593 (2002)
133. Leppa, S. and D. Bohmann: Diverse functions of JNK signaling and c-Jun in stress response and apoptosis. *Oncogene* 18, 6158 - 6162 (1999)
134. Nagata, Y., N. Takahashi, R. J. Davis, and K. Todokoro: Activation of p38 MAP kinase and JNK but not ERK is required for erythropoietin-induced erythroid differentiation. *Blood* 92, 1859 - 1869 (1998)
135. Raitano, A. B., J. R. Halpern, T. M. Hambuch, and C. L. Sawyers: The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. *Proc Natl Acad Sci U S A* 92, 11746 - 11750 (1995)
136. Hess, P., G. Pihan, C. L. Sawyers, R. A. Flavell, and R. J. Davis: Survival signaling mediated by c-Jun NH(2)-terminal kinase in transformed B lymphoblasts. *Nat Genet* 32, 201 - 205 (2002)
137. Xu, X., O. Heidenreich, I. Kitajima, K. McGuire, Q. Li, B. Su, and M. Nerenberg: Constitutively activated JNK is associated with HTLV-1 mediated tumorigenesis. *Oncogene* 13, 135 - 142 (1996)
138. Krause, D., A. Lyons, C. Fennelly, and R. O'Connor: Transient activation of Jun N-terminal kinases and protection from apoptosis by the insulin-like growth factor I receptor can be suppressed by dicumarol. *J Biol Chem* 276, 19244 - 19252 (2001)
139. Nishina, H., K. D. Fischer, L. Radvanyi, A. Shahinian, R. Hakem, E. A. Rubie, A. Bernstein, T. W. Mak, J. R. Woodgett, and J. M. Penninger: Stress-signalling kinase Sek1 protects thymocytes from apoptosis mediated by CD95 and CD3. *Nature* 385, 350 - 353 (1997)
140. Yeh, W. C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J. L. de la Pompa, D. Ferrick, B. Hum, N. Iscove, P. Ohashi, M. Rothe, D. V. Goeddel, and T. W. Mak: Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* 7, 715 - 725 (1997)
141. Rajnavolgyi, E., N. Benbernou, B. Rethi, D. Reynolds, H. A. Young, M. Magocsi, K. Muegge, and S. K. Durum: IL-7 withdrawal induces a stress pathway activating p38 and Jun N-terminal kinases. *Cell Signal* 14, 761 - 769 (2002)
142. Chen, Y. R., X. Wang, D. Templeton, R. J. Davis, and T. H. Tan: The role of c-Jun N-terminal kinase (JNK) in apoptosis induced by ultraviolet C and gamma radiation. Duration of JNK activation may determine cell death and proliferation. *J Biol Chem* 271, 31929 - 31936 (1996)
143. Ichijo, H., E. Nishida, K. Irie, P. ten Dijke, M. Saitoh, T. Moriguchi, M. Takagi, K. Matsumoto, K. Miyazono, and Y. Gotoh: Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science* 275, 90 - 94 (1997)
144. Saitoh, M., H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono, and H. Ichijo: Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 17, 2596 - 2606 (1998)
145. Hatai, T., A. Matsuzawa, S. Inoshita, Y. Mochida, T. Kuroda, K. Sakamaki, K. Kuida, S. Yonehara, H. Ichijo, and K. Takeda: Execution of apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis by the mitochondria-dependent caspase activation. *J Biol Chem* 275, 26576 - 26581 (2000)
146. Dong, C., D. D. Yang, M. Wysk, A. J. Whitmarsh, R. J. Davis, and R. A. Flavell: Defective T cell differentiation in the absence of Jnk1. *Science* 282, 2092 - 2095 (1998)
147. Dong, C., D. D. Yang, C. Tournier, A. J. Whitmarsh, J. Xu, R. J. Davis, and R. A. Flavell: JNK is required for effector T-cell function but not for T-cell activation. *Nature* 405, 91 - 94 (2000)
148. Kuan, C. Y., D. D. Yang, D. R. Samanta Roy, R. J. Davis, P. Rakic, and R. A. Flavell: The Jnk1 and Jnk2 protein kinases are required for regional specific apoptosis during early brain development. *Neuron* 22, 667 - 676 (1999)
149. Kendrick, T. S., R. J. Lipscombe, O. Rausch, S. E. Nicholson, J. E. Layton, L. C. Goldie-Cregan, and M. A. Bogoyevitch: Contribution of the membrane-distal tyrosine in intracellular signaling by the granulocyte colony-stimulating factor receptor. *J Biol Chem* 279, 326 - 340 (2004)
150. Ren, R., B. J. Mayer, P. Cicchetti, and D. Baltimore: Identification of a ten-amino acid proline-rich SH3 binding site. *Science* 259, 1157 - 1161 (1993)
151. Maeno, K., K. Sada, S. Kyo, S. M. Miah, K. Kawauchi-Kamata, X. Qu, Y. Shi, and H. Yamamura:

Adaptor protein 3BP2 is a potential ligand of Src homology 2 and 3 domains of Lyn protein-tyrosine kinase. *J Biol Chem* 278, 24912 - 24920 (2003)
152. Foucault, I., Y. C. Liu, A. Bernard, and M. Deckert: The chaperone protein 14-3-3 interacts with 3BP2/SH3BP2 and regulates its adapter function. *J Biol Chem* 278, 7146 - 7153 (2003)
153. Jevremovic, D., D. D. Billadeau, R. A. Schoon, C. J. Dick, and P. J. Leibson: Regulation of NK cell-mediated cytotoxicity by the adaptor protein 3BP2. *J Immunol* 166, 7219 - 7228 (2001)
154. Barr, R. K., T. S. Kendrick, and M. A. Bogoyevitch: Identification of the critical features of a small peptide inhibitor of JNK activity. *J Biol Chem* 277, 10987 - 10997 (2002)
155. Shklyaev, S. S., H. Namba, N. Mitsutake, G. Alipov, Y. Nagayama, S. Maeda, A. Ohtsuru, H. Tsubouchi, and S. Yamashita: Transient activation of c-Jun NH2-terminal kinase by growth factors influences survival but not apoptosis of human thyrocytes. *Thyroid* 11, 629 - 636 (2001)
156. Bain, J., H. McLauchlan, M. Elliott, and P. Cohen: The specificities of protein kinase inhibitors: an update. *Biochem J* 371, 199 - 204 (2003)
157. Fabian, M. A., W. H. Biggs, III, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lelias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar, and D. J. Lockhart: A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat Biotechnol* 23, 329 - 336 (2005)
158. Gaillard, P., I. Jeanclaude-Etter, V. Ardissone, S. Arkinstall, Y. Cambet, M. Camps, C. Chabert, D. Church, R. Cirillo, D. Gretener, S. Halazy, A. Nichols, C. Szyndralewiez, P. A. Vitte, and J. P. Gotteland: Design and synthesis of the first generation of novel potent, selective, and *in vivo* active (benzothiazol-2-yl)acetonitrile inhibitors of the c-Jun N-terminal kinase. *J Med Chem* 48, 4596 - 4607 (2005)
159. Carboni, S., A. Hiver, C. Szyndralewiez, P. Gaillard, J. P. Gotteland, and P. A. Vitte: AS601245 (1,3-benzothiazol-2-yl (2-[[2-(3-pyridinyl) ethyl] amino]-4 pyrimidinyl) acetonitrile): a c-Jun NH2-terminal protein kinase inhibitor with neuroprotective properties. *J Pharmacol Exp Ther* 310, 25 - 32 (2004)
160. Han, J., J. D. Lee, P. S. Tobias, and R. J. Ulevitch: Endotoxin induces rapid protein tyrosine phosphorylation in 70Z/3 cells expressing CD14. *J Biol Chem* 268, 25009 - 25014 (1993)
161. Han, J., J. D. Lee, L. Bibbs, and R. J. Ulevitch: A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265, 808 - 811 (1994)
162. Raingeaud, J., S. Gupta, J. S. Rogers, M. Dickens, J. Han, R. J. Ulevitch, and R. J. Davis: Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 270, 7420 - 7426 (1995)
163. Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, and A. R. Nebreda: A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. *Cell* 78, 1027 - 1037 (1994)
164. Foltz, I. N., J. C. Lee, P. R. Young, and J. W. Schrader: Hemopoietic growth factors with the exception of interleukin-4 activate the p38 mitogen-activated protein kinase pathway. *J Biol Chem* 272, 3296 - 3301 (1997)
165. Gallo, K. A. and G. L. Johnson: Mixed-lineage kinase control of JNK and p38 pathways. *Nat Rev Mol Cell Biol* 3, 663 - 672 (2002)
166. Han, J., J. D. Lee, Y. Jiang, Z. Li, L. Feng, and R. J. Ulevitch: Characterization of the structure and function of a novel MAP kinase kinase (MKK6). *J Biol Chem* 271, 2886 - 2891 (1996)
167. Raingeaud, J., A. J. Whitmarsh, T. Barrett, B. Derijard, and R. J. Davis: *Mol Cell Biol* 16, 1247 - 1255 (1996)
168. Adams, R. H., A. Porras, G. Alonso, M. Jones, K. Vintersten, S. Panelli, A. Valladares, L. Perez, R. Klein, and A. R. Nebreda: Essential role of p38alpha MAP kinase in placental but not embryonic cardiovascular development. *Mol Cell* 6, 109 - 116 (2000)
169. Mudgett, J. S., J. Ding, L. Guh-Siesel, N. A. Chartrain, L. Yang, S. Gopal, and M. M. Shen: Essential role for p38alpha mitogen-activated protein kinase in placental angiogenesis. *Proc Natl Acad Sci U S A* 97, 10454 - 10459 (2000)
170. Tamura, K., T. Sudo, U. Senftleben, A. M. Dadak, R. Johnson, and M. Karin: Requirement for p38alpha in erythropoietin expression: a role for stress kinases in erythropoiesis. *Cell* 102, 221 - 231 (2000)
171. Zarubin, T. and J. Han: Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 15, 11 - 18 (2005)
172. Ono, K. and J. Han: The p38 signal transduction pathway: activation and function. *Cell Signal* 12, 1 - 13 (2000)
173. Lee, J. C., J. T. Laydon, P. C. McDonnell, T. F. Gallagher, S. Kumar, D. Green, D. McNulty, M. J. Blumenthal, J. R. Heys, S. W. Landvatter, and J. E. Strickler, M. M. McLaughlin, I. R. Siemens, S. M. Fisher, G. P. Livi, J. R. White, J. L. Adams & P. R. Young: A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372, 739 - 746 (1994)
174. Baldassare, J. J., Y. Bi, and C. J. Bellone: The role of p38 mitogen-activated protein kinase in IL-1 beta transcription. *J Immunol* 162, 5367 - 5373 (1999)
175. Dinarello, C. A.: Inflammatory cytokines: interleukin-1 and tumor necrosis factor as effector molecules in autoimmune diseases. *Curr Opin Immunol* 3, 941 - 948 (1991)
176. Martin, M. and K. Resch: Interleukin 1: more than a mediator between leukocytes. *Trends Pharmacol Sci* 9, 171 - 177 (1988)
177. Wilson, K. P., M. J. Fitzgibbon, P. R. Caron, J. P. Griffith, W. Chen, P. G. McCaffrey, S. P. Chambers, and M. S. Su: Crystal structure of p38 mitogen-activated protein kinase. *J Biol Chem* 271, 27696 - 27700 (1996)
178. Cuenda, A., J. Rouse, Y. N. Doza, R. Meier, P. Cohen, T. F. Gallagher, P. R. Young, and J. C. Lee: SB 203580 is a specific inhibitor of a MAP kinase homologue which is stimulated by cellular stresses and interleukin-1. *FEBS Lett* 364, 229 - 233 (1995)
179. Lee, J. C., D. E. Griswold, B. Votta, and N. Hanna: Inhibition of monocyte IL-1 production by the anti-

inflammatory compound, SK&F 86002. *Int J Immunopharmacol* 10, 835 - 843 (1988)
180. Badger, A. M., J. N. Bradbeer, B. Votta, J. C. Lee, J. L. Adams, and D. E. Griswold: Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function. *J Pharmacol Exp Ther* 279, 1453 - 1461 (1996)
181. Wadsworth, S. A., D. E. Cavender, S. A. Beers, P. Lalan, P. H. Schafer, E. A. Malloy, W. Wu, B. Fahmy, G. C. Olini, J. E. Davis, J. L. Pellegrino-Gensey, M. P. Wachter, and J. J. Siekierka: RWJ 67657, a potent, orally active inhibitor of p38 mitogen-activated protein kinase. *J Pharmacol Exp Ther* 291, 680 - 687 (1999)
182. Zijlstra, J. G., J. E. Tulleken, J. J. Ligtenberg, P. de Boer, and T. S. van der Werf: p38-MAPK inhibition and endotoxin induced tubular dysfunction in men. *J Endotoxin Res* 10, 402 - 405 (2004)
183. Kuma, Y., G. Sabio, J. Bain, N. Shpiro, R. Marquez, and A. Cuenda: BIRB796 inhibits all p38 isoforms *in vitro* and *in vivo*. *J Biol Chem* 280, 19472 - 19479 (2005)
184. Pargellis, C., L. Tong, L. Churchill, P. F. Cirillo, T. Gilmore, A. G. Graham, P. M. Grob, E. R. Hickey, N. Moss, S. Pav, and J. Regan: Inhibition of p38 MAP kinase by utilizing a novel allosteric binding site. *Nat Struct Biol* 9, 268 - 272 (2002)
185. Branger, J., B. van den Blink, S. Weijer, J. Madwed, C. L. Bos, A. Gupta, C. L. Yong, S. H. Polmar, D. P. Olszyna, C. E. Hack, S. J. van Deventer, M. P. Peppelenbosch, and P. T. van der: Anti-inflammatory effects of a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. *J Immunol* 168, 4070 - 4077 (2002)
186. Branger, J., B. van den Blink, S. Weijer, A. Gupta, S. J. van Deventer, C. E. Hack, M. P. Peppelenbosch, and P. T. van der: Inhibition of coagulation, fibrinolysis, and endothelial cell activation by a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. *Blood* 101, 4446 - 4448 (2003)
187. van den Blink, B., J. Branger, S. Weijer, A. Gupta, S. J. van Deventer, M. P. Peppelenbosch, and P. T. van der: P38 mitogen activated protein kinase is involved in the downregulation of granulocyte CXC chemokine receptors 1 and 2 during human endotoxemia. *J Clin Immunol* 24, 37 - 41 (2004)
188. Davies, S. P., H. Reddy, M. Caivano, and P. Cohen: Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* 351, 95 - 105 (2000)
189. Srinivasa, S. P. and P. D. Doshi: Extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways cooperate in mediating cytokine-induced proliferation of a leukemic cell line. *Leukemia* 16, 244 - 253 (2002)
190.Bussolino, F., J. M. Wang, P. Defilippi, F. Turrini, F. Sanavio, C. J. Edgell, M. Aglietta, P. Arese, and A. Mantovani: Granulocyte- and granulocyte-macrophage-colony stimulating factors induce human endothelial cells to migrate and proliferate. *Nature* 337, 471 - 473 (1989)
191. Fuste, B., G. Escolar, P. Marin, R. Mazzara, A. Ordinas, and M. Diaz-Ricart: G-CSF increases the expression of VCAM-1 on stromal cells promoting the adhesion of CD34+ hematopoietic cells: studies under flow conditions. *Exp Hematol* 32, 765 - 772 (2004)
192. Fuste, B., R. Mazzara, G. Escolar, A. Merino, A. Ordinas, and M. Diaz-Ricart: Granulocyte colony-stimulating factor increases expression of adhesion receptors on endothelial cells through activation of p38. *Haematologica* 89, 578 - 585 (2004)
193. Lee, J. D., R. J. Ulevitch, and J. Han: Primary structure of BMK1: a new mammalian map kinase. *Biochem Biophys Res Commun* 213, 715 - 724 (1995)
194. Zhou, G., Z. Q. Bao, and J. E. Dixon: Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 270, 12665 - 12669 (1995)
195. Kamakura, S., T. Moriguchi, and E. Nishida: Activation of the protein kinase ERK5/BMK1 by receptor tyrosine kinases. Identification and characterization of a signaling pathway to the nucleus. *J Biol Chem* 274, 26563 - 26571 (1999)
196. Kato, Y., R. I. Tapping, S. Huang, M. H. Watson, R. J. Ulevitch, and J. D. Lee: Bmk1/Erk5 is required for cell proliferation induced by epidermal growth factor. *Nature* 395, 713 - 716 (1998)
197. Chao, T. H., M. Hayashi, R. I. Tapping, Y. Kato, and J. D. Lee: MEKK3 directly regulates MEK5 activity as part of the big mitogen-activated protein kinase 1 (BMK1) signaling pathway. *J Biol Chem* 274, 36035 - 36038 (1999)
198. Marinissen, M. J., M. Chiariello, M. Pallante, and J. S. Gutkind: A network of mitogen-activated protein kinases links G protein-coupled receptors to the c-jun promoter: a role for c-Jun NH2-terminal kinase, p38s, and extracellular signal-regulated kinase 5. *Mol Cell Biol* 19, 4289 - 4301 (1999)
199. Abe, J., M. Takahashi, M. Ishida, J. D. Lee, and B. C. Berk: c-Src is required for oxidative stress-mediated activation of big mitogen-activated protein kinase 1. *J Biol Chem* 272, 20389 - 20394 (1997)
200. Kato, Y., T. H. Chao, M. Hayashi, R. I. Tapping, and J. D. Lee: Role of BMK1 in regulation of growth factor-induced cellular responses. *Immunol Res* 21, 233 - 237 (2000)
201. Yan, L., J. Carr, P. R. Ashby, V. Murry-Tait, C. Thompson, and J. S. Arthur: Knockout of ERK5 causes multiple defects in placental and embryonic development. *BMC Dev Biol* 3, 11 (2003)
202. Sohn, S. J., B. K. Sarvis, D. Cado, and A. Winoto: ERK5 regulates embryonic angiogenesis and acts as a hypoxia-sensitive repressor of vascular endothelial growth factor expression. *J Biol Chem* 277, 43344 - 43351 (2002)
203. Regan, C. P., W. Li, D. M. Boucher, S. Spatz, M. S. Su, and K. Kuida: Erk5 null mice display multiple extraembryonic vascular and embryonic cardiovascular defects. *Proc Natl Acad Sci U S A* 99, 9248 - 9253 (2002)
204. Hii, C. S., D. S. Anson, M. Costabile, V. Mukaro, K. Dunning, and A. Ferrante: Characterization of the MEK5-ERK5 module in human neutrophils and its

relationship to ERK1/ERK2 in the chemotactic response. *J Biol Chem* 279, 49825 - 49834 (2004)

Abbreviations: G-CSF: Granulocyte Colony-Stimulating Factor, G-CSFR Granulocyte Colony-Stimulating Factor Receptor, MAPK: Mitogen-Activated Protein Kinase, ERK: Extracellular Signal-Regulated Kinase, JNK: c-Jun N-terminal Kinase, Y: tyrosine, PI3K: Phosphatidylinositol 3'Kinase, MAPKKK: MAPK kinase kinase, MAPKK: MAPK kinase, PTB domain: Phosphotyrosine Binding domain, EGF: Epidermal Growth Factor, NGF: Nerve Growth Factor, FGF: Fibroblast Growth Factor, OSM: Oncostatin M, IL: interleukin, SH2: Src Homology 2, SH3: Src Homology 3, GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor

Key Words: ERK 1/2, c-Jun, N-terminal, Kinase, p38, Src Homology, Oncostatin, Epidermal Growth Factor, Nerve Growth Factor, Fibroblast Growth Factor, ERK5, MAPKKK, MAPK Pathways, Granulocyte Colony Stimulating Factor, GCSF Receptor, Proliferation, Neutrophil, Function, Review

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