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REVIEW

MAP kinase signalling pathways in cancer

AS Dhillon[1], S Hagan[1], O Rath[1] and W Kolch[1,2]

[1]The Beatson Institute for Cancer Research, Bearsden, Glasgow, UK and [2]Sir Henry Wellcome Functional Genomics Facility, University of Glasgow, Glasgow, UK

Cancer can be perceived as a disease of communication between and within cells. The aberrations are pleiotropic, but mitogen-activated protein kinase (MAPK) pathways feature prominently. Here, we discuss recent findings and hypotheses on the role of MAPK pathways in cancer. Cancerous mutations in MAPK pathways are frequently mostly affecting Ras and B-Raf in the extracellular signal-regulated kinase pathway. Stress-activated pathways, such as Jun N-terminal kinase and p38, largely seem to counteract malignant transformation. The balance and integration between these signals may widely vary in different tumours, but are important for the outcome and the sensitivity to drug therapy.
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Introduction

Mitogen-activated protein kinase (MAPK) pathways are evolutionarily conserved kinase modules that link extracellular signals to the machinery that controls fundamental cellular processes such as growth, proliferation, differentiation, migration and apoptosis. MAPK pathways are comprised of a three-tier kinase module in which a MAPK is activated upon phosphorylation by a mitogen-activated protein kinase kinase (MAPKK), which in turn is activated when phosphorylated by a MAPKKK (Figure 1). To date six distinct groups of MAPKs have been characterized in mammals; extracellular signal-regulated kinase (ERK)1/2, ERK3/4, ERK5, ERK7/8, Jun N-terminal kinase (JNK)1/2/3 and the p38 isoforms $\alpha/\beta/\gamma$(ERK6)/δ (Schaeffer and Weber, 1999; Chen *et al*., 2001b; Kyriakis and Avruch, 2001; Krens *et al*., 2006). The current consensus is that tumorigenesis requires deregulation of at least six cellular processes (Johnson *et al*., 1996), and that cancer cells have to acquire the following capabilities: independence of proliferation signals, evasion of apoptosis, insensitivity to anti-growth signals, unlimited replicative potential, the ability to invade and metastasize and to attract and sustain angiogenesis for nutrient supply (Hanahan and Weinberg, 2000). To this we may add acquisition of drug resistance and avoidance of oncogene induced senescence. Abnormalities in MAPK signalling impinge on most, if not all these processes, and play a critical role in the development and progression of cancer. As the literature on MAPK pathways and cancer is huge and includes comprehensive recent reviews (Downward, 2003; Wellbrock *et al*., 2004; Kolch, 2005; Bradham and McClay, 2006; Galabova-Kovacs *et al*., 2006; Kohno and Pouyssegur, 2006; Torii *et al*., 2006), we will take the liberty of a more subjective view and discuss emerging areas and interesting questions in the field.

Correspondence: Dr AS Dhillon, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow G61 1BD, UK or Professor W Kolch, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Glasgow G61 1BD, UK.
E-mails: a.dhillon@beatson.gla.ac.uk or wkolch@beatson.gla.ac.uk

The ERK pathway

The ERK pathway is the best studied of the mammalian MAPK pathways, and is deregulated in approximately, one-third of all human cancers. Historically, ERK signalling was synonymous with cell proliferation but it is now clear that that deregulation of this pathway is linked to many other aspects of the tumour phenotype. In the ERK MAPK module, ERK (ERK1 and ERK2) is activated upon phosphorylation by MEK (MEK1 and MEK2), which is itself activated when phosphorylated by Raf (Raf-1, B-Raf and A-Raf). ERK signalling is activated by numerous extracellular signals. The pathway whereby growth factors and mitogens activate ERK signalling is of particular relevance to cancer. In this pathway, ligand-mediated activation of receptor tyrosine kinases triggers guanosine triphosphate (GTP) loading of the Ras GTPase, which can then recruit Raf kinases to the plasma membrane for activation. Most cancer-associated lesions that lead to constitutive activation of ERK signalling occur at these early steps of the pathway, namely, overexpression of receptor tyrosine kinases, activating mutations in receptor tyrosine kinases, sustained autocrine or paracrine production of activating ligands, *Ras* mutations and *B-Raf* mutations (Figure 2). However, there is also amplification or deregulation of its nuclear transcription factor targets, most notably *myc* and AP-1. In addition, cancer cells may switch the repertoire of extracellular matrix receptors they express to one that favours the

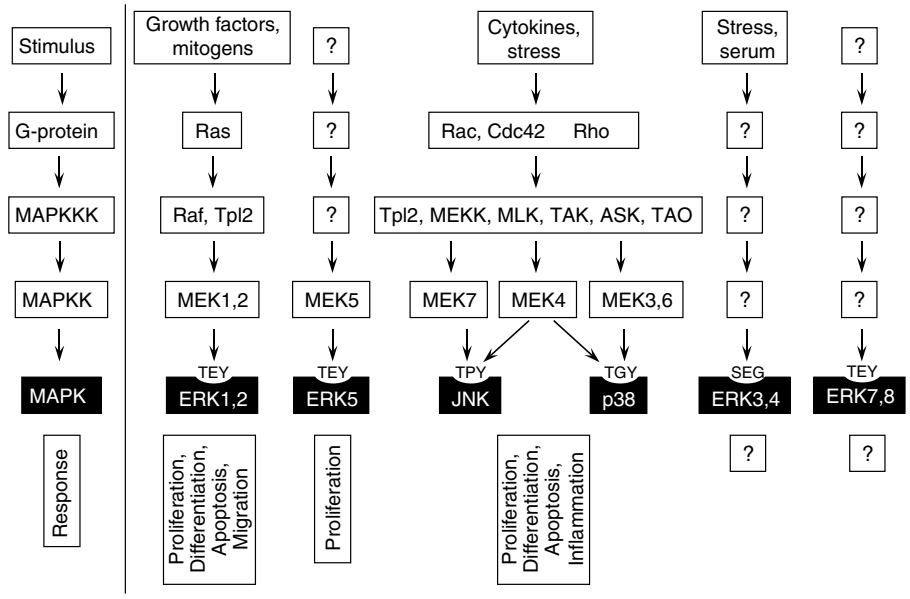

Figure 1 Schematic overview of MAPK pathways. See text for details.

EGFR overexpression	• Most carcinomas (>50%)
ERBB2 overexpression	• Breast (30%)
RAS mutation	• Pancreas (90%) • Lung adenocarcinoma (35%) (non-small cell) • Thyroid; follicular (55%) • Thyroid; undifferentiated papillary (60%) • Seminoma (45%) • Melanoma (15%) • Bladder (10%) • Liver (30%) • Kidney (10%) • Myelodysplastic syndrome (40%) • Acute myelogenous leukemia (30%)
BRAF mutation	• Melanoma (66%) • Colorectal (12%)

Figure 2 Cancer-associated lesions in the ERK-signalling pathway in cancer, adapted from Downward (2003).

transmission of pro-growth signals. Such growth promoting integrins can activate Ras signalling (Giancotti and Ruoslahti, 1999). Thus, the fact that deregulation of this pathway in cancer occurs at several levels underlines its importance. The high frequency of activating mutations centred around the Ras–Raf axis suggests that this is the regulatory hotspot of the pathway. Indeed mathematical modelling predicts that Ras and Raf activation are very sensitive points of regulation that can determine the overall activation profile (Orton *et al*., 2005). Further, the fact that Ras and B-Raf mutations rarely occur in the same tumour cell can be taken as indication that Raf is a main effector pathway of Ras in human carcinogenesis. However, an alternative explanation is that Ras and B-Raf mutations could be synthetic lethal, and there is some evidence for that showing that co-expression of mutant B-Raf with mutant N-Ras induces senescence (Petti *et al*., 2006).

Ras

Ras GTPases act as molecular switches that control the activity of many signalling pathways. Activating mutations in *K-Ras* and *N-Ras* occur in varying frequencies in different types of cancer and have been recently reviewed (Downward, 2003; Sebolt-Leopold and Herrera, 2004). These mutations, invariably found at codons 12, 13 or 61, prevent efficient GTP hydrolysis, rendering Ras in an active, GTP-bound state. In this conformation, Ras oncogenes can bind and activate their effectors including Raf. Although initially thought to occur mainly at the plasma membrane, there is increasing evidence that isoform-specific Ras signalling can take place at different cellular compartments and within different regions of the plasma membrane (Hancock, 2003; Hancock and Parton, 2005; Philips, 2005; Mor and Philips, 2006). Such compartmentalization and trafficking of endogenous Ras oncogenes is likely to play an important role in regulating downstream signalling processes involved in tumorigenesis and is a subject that requires further investigation. For instance, one could envision drugs that selectively target oncogenic functions of Ras by affecting its subcellular localization. It would be interesting to analyse whether the subcellular localization of mutant Ras proteins is altered in tumours.

GTP-loaded Ras also recruits other molecules that play an important role nucleating an active signalling complex that is competent in activating ERK (Kolch, 2005). These complexes include scaffolds such as KSR (Therrien *et al*., 1996) and SUR-8/SHOC-2 (Li *et al*., 2000) which modulate the activation of Raf by Ras. Although no mutations in these scaffolds have been reported in human

cancer, KSR knock-out mice have a reduced tumour susceptibility (Nguyen *et al*., 2002) pointing to a role of these proteins in cancer development.

The importance of Ras proteins in a variety of tumours suggested that they would be good therapeutic targets (Sebolt-Leopold and Herrera, 2004). For Ras to function as signal transducer, it has to associate with the plasma membrane. This step requires isoprenylation (farnesylation or geranylation) near the Ras C-terminus. Consequently, Ras was targeted isoprenylation inhibitors. However, in the clinic these inhibitors were largely disappointing (Beeram *et al*., 2003; Zhu *et al*., 2003). The reason is not entirely clear. In part this may be related to observations suggesting that the anti-tumour effects of farnesylation inhibitors are due to effects on Rho rather than due to Ras inhibition (Du and Prendergast, 1999). Further, farnesylation inhibitors do not distinguish between normal and mutant Ras, and as normal Ras can counteract the transforming action of mutant Ras (To *et al*., 2006), they may remove accelerator and brakes at the same time. Thus, more recent efforts have been focussed on the disrupting signalling downstream of Ras.

Raf regulation

Raf kinases are direct effectors of Ras and lie at the apex of the ERK pathway kinase module. The structures of the three Raf proteins are similar, but there are salient differences how they are activated (O'Neill and Kolch, 2004; Wellbrock *et al*., 2004). Though sharing several common structural characteristics, the three mammalian Raf isoforms differ considerably in their modes of regulation, tissue distributions and abilities to activate MEK (Wellbrock *et al*., 2004). Genetic ablation of the different Raf isoforms in mice suggests that they serve mainly non-redundant roles *in vivo* (O'Neill and Kolch, 2004; Galabova-Kovacs *et al*., 2006). Once bound to Ras, Raf kinases are activated by a complex sequence of events involving phosphorylation, protein–protein and protein–lipid interactions (Dhillon and Kolch, 2002; Chong *et al*., 2003; Wellbrock *et al*., 2004). These events increase the catalytic ability of Raf both by neutralising autoinhibition and facilitating activation of the kinase domain. Raf-1 activation involves a complex series of changes in phosphorylation, which entail the dephosphorylation of an inhibitory site, S259, and the phosphorylation of the N-region including a critical activating site, S338, as well as phosphorylation of the activation loop for maximal activation. These sites are conserved in A-Raf, and activation seems to follow a similar pattern to Raf-1. However, B-Raf has already a negative charge in the N-region due to twin aspartic acids and the equivalent of Raf-1 S338 is constitutively phosphorylated. Additionally, Ras alone is sufficient to activate B-Raf, whereas Raf-1 requires other factors in addition. However, it is still unclear which of the Raf isoforms is required to activate ERK, and this may be different dependent on the cellular context and the stoichiometries of Raf isoforms (Galabova-Kovacs *et al*., 2006).

B-raf mutations and raf-1/b-raf heterodimers

B-Raf has attracted enormous interest, as the *b-raf* gene is found mutated in 66% of malignant melanomas (Davies *et al*., 2002), and at a lower frequency in many other human malignancies, including colon cancer, papillary thyroid cancer and serous ovarian cancer. This discovery has firmly established the involvement of Raf kinases in cancer. The most common mutation (ca. 90%) is a V600E change in the activation loop that induces the constitutive activation of catalytic activity (Wan *et al*., 2004). Curiously, in melanoma this mutation is rare in unexposed or chronically sun-damaged skin, but frequent in skin with intermittent sun exposure and often accompanied by amplification of the mutant allele (Maldonado *et al*., 2003). The importance of localization is underlined by the observation that B-Raf mutations do not occur in melanomas of the uvea (Spendlove *et al*., 2004). Further, the frequency of B-Raf mutations in melanoma is positively linked with genetic variants of the melanocortin-1 receptor (Landi *et al*., 2006) in melanocytes, and in colorectal carcinoma (CRC) with microsatellite instability (MSI). Although it has no bearing on the good prognosis of MSI-positive tumours, it is associated with poor prognosis in microsatellite-stable cancers (Samowitz *et al*., 2005). Further, B-Raf mutations in CRC correlated with a high level of multiple promoter methylation at CpG islands, whereas K-Ras mutation only showed a weak association (Nagasaka *et al*., 2004). These findings suggest that B-Raf mutations are promoted by complex genetic interactions rather than physicochemical mechanisms. They also could indicate that B-Raf mutations are lethal unless a certain genetic and biochemical microenvironment permits such cells to survive.

Studies into the mechanisms of oncogenic B-Raf signalling have highlighted novel mechanisms by which, Raf kinases activate MEK-ERK signalling that in part differ from the classical Ras pathway. The V600E mutation drastically elevates B-Raf kinase activity and its ability to activate the ERK pathway, as do most other of the cancer-associated mutations (Garnett and Marais, 2004). Curiously, a few mutations do not elevate B-Raf kinase activity, yet are still able to activate MEK-ERK signalling (Wan *et al*., 2004). This puzzle gave rise to recent discoveries that B-Raf heterodimerizes with Raf-1 and can signal through Raf-1 (Garnett *et al*., 2005; Rushworth *et al*., 2006). These studies showed that Raf-1/B-Raf heterodimerization is part of the physiological activation mechanism and contribute an important part to activation of the ERK pathway by low activity B-Raf mutants, but differ in details. Rushworth *et al*. (2006) showed that Raf-1/B-Raf heterodimerization was stimulated by mitogens, enhanced by 14-3-3, and that in the context of the heterodimer either Raf isoform could activate the other. Direct measurements of the kinase activities of the heterodimers showed that despite its low abundance it contributed to a substantial level of ERK activity. The kinase activity of the Raf-1/B-Raf heterodimer towards MEK was considerably higher than the activity of B-Raf

or Raf-1 on their own suggesting the intriguing possibility that the heterodimer may be the main MEK activator, whereas the non-heterodimeric isoforms may work in other pathways. The Marais group found that the activation of Raf in the heterodimer is one way, that is, B-Raf can activate Raf, but not *vice versa*, and that this type of activation of Raf-1 by B-Raf mutants occurs through Raf-1 activation loop phosphorylation independently of Ras (Garnett *et al*., 2005). This would indicate a profound difference between the physiological activation of Raf-1 that seems to obligatory require Ras (Marais *et al*., 1998), and the activation of Raf-1 by B-Raf mutants, by implication suggesting that a tumour-specific mechanism for Raf-mediated MEK activation exists. This may explain why tumour cells with *B-Raf* mutations apparently are exquisitely sensitive to MEK inhibition whereas tumour cells with Ras mutations are rather resistant (Solit *et al*., 2006). Conceptually, this is surprising as it would indicate that Ras can transform cells without the need to activate MEK, contradicting the tenet that B-Raf is the main effector of Ras transformation. Thus, the role of Raf-1/B-Raf dimerization clearly is of high interest and relevance for carcinogenesis, and warrants further investigations in order to draw firm conclusions about the molecular mechanism. In this context it is interesting to note that normal melanocytes seem to preferentially use B-Raf to activate ERK, because Raf-1 activity is suppressed by cyclic AMP-dependent kinase (PKA) signalling. However, *Ras* mutations in melanoma cells uncouple PKA from Raf-1 regulation causing a switch from B-Raf to Raf-1 signalling and ERK activation becoming dependent on Raf-1 (Dumaz *et al*., 2006). It would be interesting to investigate whether this switch also includes changes in Raf-1/B-Raf heterodimerization and whether PKA regulates heterodimer formation.

Interestingly, *B-Raf* mutations (and less frequently activating MEK mutations) were also discovered in cardio–facio–cutaneous (CFC) syndrome (Rodriguez-Viciana *et al*., 2006), a hereditary disease hallmarked by mental retardation, congenital heart defects and abnormalities of facial structure and skin. Although the CFC mutations activate the kinase activity of B-Raf comparable to the oncogenic V600E mutation, CFC patients are not predisposed to cancer. These results and the results from Raf isoform knock-out mice (Galabova-Kovacs *et al*., 2006) raise a number of important questions pertaining to the regulation of B-Raf activation and signalling, including for instance whether B-Raf mutants are still regulated, and whether B-Raf can signal to different downstream targets depending on cell type or tissue-specific modifier proteins. The existence of suppressors of mutant B-Raf is suggested by the finding that $>80\%$ of benign naevi contain oncogenic B-Raf mutations without ever progressing to melanoma (Pollock *et al*., 2003). A candidate for such a suppressor is RKIP which was originally isolated as a physiological inhibitor of Raf-1 mediated MEK phosphorylation (Yeung *et al*., 1999). RKIP expression is reduced in melanoma cells with mutated B-Raf, and reconstitution of its expression to physiological levels suppressed the activity of the ERK pathway to normal levels and blocked cell invasion into matrigel (Schuierer *et al*., 2004). There are probably multiple inhibitory mechanisms that must be circumvented including escape from senescence. High-level signalling by mutated B-Raf can induce senescence both in human melanocytes and in congenital naevi thus preventing the mutation to induce malignant progression (Michaloglou *et al*., 2005).

Raf-1 mutations

In contrast to B-Raf, mutations in *Raf-1* are very rare, and no *A-Raf* mutations were found. Four Raf-1 mutations were detected in 545 established cancer cell lines (Emuss *et al*., 2005), but is not entirely clear whether these are polymorphisms. Only one mutant had elevated kinase activity, but failed to transform cells. Interestingly, mutating the residue equivalent to B-Raf V600E also failed to produce a transforming Raf-1 protein unless a negative charge was introduced into the N-region. These data show that it takes two mutations to convert Raf-1 into a transforming protein by the same mechanism as B-Raf, and this may explain why B-Raf is the preferred target for mutation in cancer. However, truncation or even single-point mutations can confer transforming activity onto Raf-1 (Dhillon and Kolch, 2002; Wellbrock *et al*., 2004) indicating that there may be other reasons for the preference of B-Raf mutations in cancer. Two mutations in the Raf-1 kinase domain have been found in acute myeloid leukaemia (Zebisch *et al*., 2006). This disease features ERK activation in more than 50% of cases, but the frequency of Raf-1 mutations was less than 1/400. One mutation activated Raf-1 whereas the other did not, although both mutants could enhance survival and induce transformation in *in vitro* assays, indicating that the role of Raf-1 in cancer may not rely solely on its kinase activity, but also involve kinase independent functions. These non-catalytic Raf-1 functions include the counteraction of apoptosis by suppressing the proapoptotic kinases ASK-1 (Chen *et al*., 2001a) and MST2 (O'Neill and Kolch, 2004), and the membrane expression of Fas (Piazzolla *et al*., 2005), as well as the regulation of ROKα to stimulate cell migration (Ehrenreiter *et al*., 2005).

MEK and ERK signalling

Activated Raf activates MEK1 and MEK2 by phosphorylating serines 218 and 222 in the activation loop. The three Raf isoforms differ in their abilities to activate MEK1 and MEK2; B-Raf is the strongest MEK kinase followed by Raf-1. A-Raf is a weak MEK activator and preferentially activates MEK1, whereas Raf-1 activates both MEK1/2 with equal efficiency (Wu *et al*., 1996; Marais *et al*., 1997). Raf-1 has two separate MEK-binding sites, with phosphorylation of sites in the N-region strongly enhancing MEK binding (Xiang *et al*., 2002). The constitutive negative charge of this region in B-Raf and may explain the better binding and activation of MEK by B-Raf (Emuss *et al*., 2005). In addition, the ability of Raf to efficiently activate MEK in cells is likely

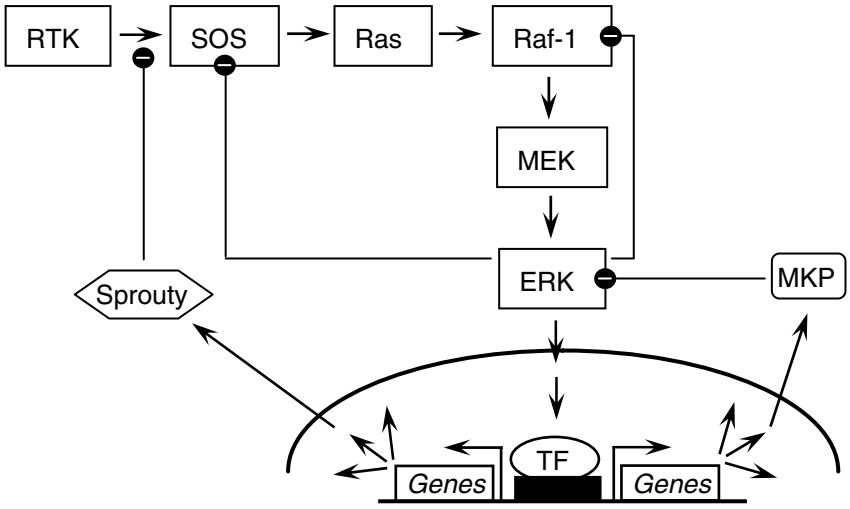

Figure 3 Negative feedback loops in the ERK pathway. Activated ERK can inhibit Raf-1 by direct phosphorylation (Dougherty *et al*., 2005). It also can interfere with the coupling of the Ras exchange factor SOS to receptors by inducing inhibitory phosphorylation directly or indirectly via $p90^{RSK2}$ (Dong *et al*., 1996; Douville and Downward, 1997). Activated ERK accumulates in the nucleus inducing the transcription of MKPs, which dephosphorylate ERK activation sites (Keyse, 2000), and Sprouty family proteins, which interfere with Ras and Raf activation (Mason *et al*., 2006).

to be influenced by the presence of scaffolds such as KSR (Morrison and Davis, 2003). MEK is also phosphorylated at S298 by PAK1, an event that may facilitate its coupling to Raf (Frost *et al*., 1997; Coles and Shaw, 2002). In addition, an inhibitory phosphorylation site on MEK, S212 was recently reported (Gopalbhai *et al*., 2003).

Active ERKs phosphorylate numerous cytoplasmic and nuclear targets, including kinases, phosphatases, transcription factors and cytoskeletal proteins (Yoon and Seger, 2006). ERK signalling can, depending on the particular cell type, regulate processes such diverse as proliferation, differentiation, survival, migration, angiogenesis and chromatin remodelling (Dunn *et al*., 2005; Yoon and Seger, 2006). A key question is how ERK can perform these different roles with high specificity and reliability. In part at least, these properties may be linked to temporal differences in the strength and localization of ERK within the cell (Murphy and Blenis, 2006). Recent studies have shown that different expression levels and phosphorylation of early gene products induced by ERK signalling, such as Fos, Jun, Myc and Egr-1 may function as sensors for ERK signalling dynamics (Murphy *et al*., 2002, 2004). Sustained, but not transient ERK signalling, promotes phosphorylation and stabilization of such genes, thereby promoting cell-cycle entry. Sustained ERK signalling not only promotes to accumulation of genes required for cell-cycle entry such as cyclin D1, it can also repress the expression of genes which inhibit proliferation (Yamamoto *et al*., 2006). In addition to temporal factors, the ERK signalling also influences cellular processes by varying signalling its signalling strength. High levels of ERK signalling can lead to cell-cycle arrest by inducing the expression of CDK-inhibitor protein such as p21 and p27 (Sewing *et al*., 1997; Woods *et al*., 1997; Mirza *et al*., 2004). To continue to proliferate, certain tumour cells utilize mechanisms, such as elevating Rho signalling or constitutively activating Akt, to counteract the ERK-mediated induction of these CDK inhibitor proteins (Olson *et al*., 1998; Sahai *et al*., 2001; Coleman *et al*., 2004; Mirza *et al*., 2004).

ERK signalling also plays a role in the disrupting the anti-proliferative effects of ligands such as transforming growth factor beta (TGFβ). For example, activated N-Ras induces the cytoplasmic mislocalization of p27 via the Ral-GEF pathway, leading to the disruption of TGFβ-mediated Smad nuclear translocation Accumulating evidence also suggest that the expression of different feedback inhibitors the ERK pathway is deregulated in cancer (Figure 3). These include MAP kinase phophatases (MKPs) and Sprouty family members (Miyoshi *et al*., 2004; Tsavachidou *et al*., 2004; Bloethner *et al*., 2005; Tsujita *et al*., 2005; Fong *et al*., 2006). Interestingly, ERK signalling is restrained even in transformed cells through multiple negative feedback loops. It seems that the balance between ERK activity and negative feedback is more important than the absolute level of ERK activation. This could be due to the need to avoid that high level ERK signalling induces cell-cycle arrest (Sewing *et al*., 1997; Woods *et al*., 1997; Mirza *et al*., 2004), but more provocatively also may indicate that some of these inhibitors actually may contribute to tumorigenesis.

The ERK pathway as a drug target

Because of its importance in cancer the ERK pathway has been a focus for drug discovery for almost 15 years with Ras, Raf and MEK as the main targets (Downward, 2003; Kohno and Pouyssegur, 2006). Currently, the most promising drug targeting Raf kinases is Sorafenib (BAY43-9006). In melanomas where B-Raf mutations are a major driver of tumorigenesis,

monotherapy with Sorafenib is well tolerated but has little or no antitumour activity (Eisen *et al*., 2006). However, Sorafenib is not specific for Raf kinases as there is growing evidence that a significant part of its antitumour activity is due to its effect on the platelet-derived growth factor (PDGF) and vascular endothelial growth factor receptor 2 receptor (Wilhelm and Chien, 2002). This may explain why it is efficacious in renal cancer which is well vascularized and hallmarked by a dysregulation of vascular endothelial growth factor (VEGF) and PDFG receptors (Gollob, 2005). A variation on this theme are heat-shock protein 90 (HSP90) inhibitors (Chiosis, 2006; Sharp and Workman, 2006). HSP90 is an obligatory chaperone for several signalling proteins including Raf kinase, Akt and EGF receptors. Disruption of binding to HSP90 leads to the degradation of these proteins. This is exploited by drugs like geldanamycin that are in clinical trials with results that support the validity of this approach (Sharp and Workman, 2006). The probably best explored strand is MEK inhibitors. CI-1040, the first MEK inhibitor to enter clinical trials, was well tolerated, but did not provide sufficient anti-tumour activity to be taken forward. Hopes are now on PD0325901, a second-generation MEK1/2 inhibitor, with improved pharmaceutical and pharmacological properties (Rinehart *et al*., 2004). The Raf and MEK inhibitors illustrate two polarized philosophies of drug discovery. Sorafenib is pluripotent inhibitor, whereas PD0325901 is highly selective. The question is which one to go for? Empirically most cancer drugs are used in combination therapies, and given the multiple aberrations found in cancer cells, it is pragmatic and seems an inherent advantage to hit several deviant pathways simultaneously with one drug. On the other hand, the conceptually more pleasing approach is to develop highly selective inhibitors that can be combined as required for the treatment of different cancers and individual patients. However, pluripotent inhibitors are difficult to design and develop purposefully as it is almost impossible to optimize several features in parallel. An even more formidable task may be to develop highly selective inhibitors and then figure out in which combinations to deploy them for maximum effect. We have no systematic rational framework in neither of these areas and hence it seems sensible to pursue both approaches.

Another interesting concept to generate specificity and minimize side effects is to target downstream effectors thereby ensuring that only certain functions are eliminated. With its multiple effectors, the ERK pathway provides a rich target area. It would go beyond the scope of this review to discuss details, but in addition to cell proliferation, other relevant potential targets downstream of ERK play key roles in angiogenesis, cell migration, invasion and metastasis (Reddy *et al*., 2003; Giehl, 2005). One important mechanism whereby ERK signalling may promote a more malignant phenotype is by disrupting Rho signalling pathways (Sahai and Marshall, 2002). For example, ERK-mediated upregulation of the Fra-1, a component of the AP-1 transcription factor complex deregulates Rho signalling in colon carcinoma cells to promote cell motility (Vial *et al*., 2003; Pollock *et al*., 2005). ERK activity can also promote endothelial cell survival and blood vessel sprouting via the suppression of Rho-kinase signalling (Mavria *et al*., 2006). In addition to its effects on Rho signalling, ERK can phosphorylate a number of proteins involved in cell migration including MLCK, calpain, FAK and paxillin (Huang *et al*., 2004) as well as regulate the expression of proteases involved in basement membrane degradation (Reddy *et al*., 2003). In mouse models, oncogenic Ras expression has been linked to increased VEGF production, which promotes angiogenesis and contributes to subsequent tumour maintenance (Chin *et al*., 1999; Eves *et al*., 2006). Direct phosphorylation of HIF-1α and Sp1 by ERK1/2 has been shown to induce transcription of VEGF, a key regulator of angiogenesis (Richard *et al*., 1999). Sustained activation of ERK pathway is also a necessary step in basic fibroblast growth factor-induced angiogenesis (Eliceiri *et al*., 1998).

Stress-activated MAPK pathways

Many MAPK pathways participate in stress signalling. In general, stress activated MAPKs cascades feature a large number of MAPKKKs probably reflecting that stress comes in many forms and unlike growth factors has few specific receptors. Thus, there seems to be a larger input network necessary for the sensing and processing of stress signals. Cancer cells are exposed to various stress conditions including hypoxia, detachment from substrate, inflammation and metabolic stress arising from dysregulation of energy production. Added to this are genotoxic and pharmacological stress during chemotherapy or radiotherapy. Thus, an important part for stress-activated kinases in cancer is emerging, mainly in modulatory roles that impinge on inflammation, DNA damage response and apoptosis. Generally, their effect is anti-proliferative and proapoptotic, but dependent on the cellular context they also may contribute to tumorigenesis.

The JNK pathway

The JNK family of MAP kinases are predominantly activated by cytokines, UV radiation, growth factor deprivation, DNA-damaging agents, certain G-protein coupled receptors and serum (Weston and Davis, 2002). The family is encoded by three genes – *Jnk1*, *Jnk2* and *Jnk3*. *Jnk 1* and *2* are ubiquitously expressed, whereas *Jnk3* expression is restricted to the brain, heart and testis. Alternative splicing of these genes creates a total of 10 JNK isoforms. JNK activation requires dual phosphorylation on tyrosine and threonine residues at a distinctive TPY motif, a reaction is catalysed by MEK4 and MEK7. MEK4 and MEK7 are themselves phosphorylated and activated by several MAPKKKs, including MEKK1–4, MLL2 and 3, YTpl-2, DLK, TAO1 and 2, TAK1 and ASK1 and 2. Like p38, JNK

are translocate relocate from the cytoplasm to the nucleus following activation. A major substrate for JNK is the transcription factor c-Jun, which when phosphorylated at serines 63 and 73, results in the enhancement of AP-1 transcriptional activity (Adler *et al*., 1992). JNKs can also phosphorylate several other transcription factors, including ATF-2, NF-ATc1, HSF-1 and STAT3 (Ip and Davis, 1998). The localization of active JNK is not restricted to the nucleus but relatively little is known about the nature of cytoplasmic JNK substrates.

In response to stresses such as UVB radiation, oxidative stress and DNA-damage, JNK binds to and phosphorylates p53 (Wu, 2004). Depending on the site phosphorylated, this can result in an increase in p53 transcriptional activity and p53 stabilization (Buschmann *et al*., 2000, 2001; She *et al*., 2002; Cheng *et al*., 2003). JNK has also been reported to regulate p53 stability in the absence of stress by a MDM2-dependent mechanism (Fuchs *et al*., 1998).

JNK activity and phosphorylation of c-Jun has been reported to play a critical role in Ras-induced tumorigenesis and Ras and c-Jun cooperate in cellular transformation (Smeal *et al*., 1991; Kennedy and Davis, 2003). Ras induces phosphorylation of c-Jun on the same sites as JNK and c-Jun-deficient fibroblasts are resistant to Ras-induced transformation (Schutte *et al*., 1989; Derijard *et al*., 1994; Johnson *et al*., 1996). One important function of c-Jun appears to be the transcriptional repression of the p53 gene (Schreiber *et al*., 1999; Eferl *et al*., 2003). In contrast to these findings, studies on JNK1/2-null cells have shown that JNK is not required for Ras-induced transformation and tumorigenesis *in vivo*. Instead JNK may have a tumour suppressive function that is linked to its ability to promote apoptosis (Kennedy *et al*., 2003). JNK inhibitors have been considered for cancer therapy because of their ability to interfere with DNA repair in response to genotoxic drugs (Vasilevskaya and O'Dwyer, 2003). However, as these inhibitors also may prevent apoptosis, their usefulness is unclear.

In contrast to JNK signalling, activation of nuclear factor kappa B (NF-κB) signalling can lead to the suppression of apoptosis (Bubici *et al*., 2004). In cancer, JNK and NF-κB signalling often play opposing roles, with JNK activation being tumour suppressive whereas activation of NF-κB can prevent oncogene-induced apoptosis (Orlowski and Baldwin, 2002; Franzoso *et al*., 2003; Kennedy and Davis, 2003; Kucharczak *et al*., 2003). In response to tumour necrosis factor alpha (TNFα), the anti-apoptotic effect of NF-κB has been shown to be mediated by the induction of genes that can repress JNK activity (Javelaud and Besancon, 2001; Tang *et al*., 2002). Oncogenes such as Ras are potent inducers of sustained JNK activation and activation of NF-κB may be required to suppress JNK-induced apoptosis during tumorigenesis (Davis, 2000; Bubici *et al*., 2004). Thus, inhibition of NF-κB activity may be a useful avenue to promote apoptosis in such cells *via* a JNK-dependent mechanism.

The p38 pathway

In mammals, p38 isoforms are strongly activated by environmental stresses and inflammatory cytokines. p38 is required for expression of TNFα and interleukin-1 during inflammatory responses and most stimuli that activate p38 also induce expression of the p38 protein (Zarubin and Han, 2005). Characterization of the function of p38 has been facilitated by the anti-inflammatory drug SB203580, an inhibitor of p38 (Kyriakis and Avruch, 2001).

The four vertebrate isoforms of p38, α, β and γ (ERK6) and δ are characterized by the presence of the conserved Thr-Gly-Tyr (TGY) phosphorylation motif in their activation loop (Kumar *et al*., 2003). This motif is phosphorylated by MEK3 and MEK6, which themselves are activated by various MAPKKKs that are induced by physical and chemical stresses, such as oxidative stress, hypoxia, X-ray and UV irradiation and cytokines. In some instances p38 can also be activated by MEK4, a kinase that is better known as an activator of JNK. Once active, p38 proteins can translocate to from the cytosol to the nucleus where they phosphorylate serine/threonine residues of their many substrates. In addition to its role in stress responses, the p38 pathway also plays a role in the regulation of apoptosis, cell cycle progression, growth and differentiation. This is due, in part, to the ability of a broad range of extracellular stimuli such growth factors (such as GM-CSFD, fibroblast growth factor, insulin-like growth factor 1, PDGF and nerve growth factor) and hormones that activate this pathway. Such stimuli feed into this pathway by activating different MAPKKKs, including TAK1, ASK1/2, DLK, MEKK4, TAO1/2/3 and MLK2/3 (Zarubin and Han, 2005; Krens *et al*., 2006).

Analysis of the phenotype of mice disrupted in both the MEK3 and MEK6 genes or the p38α gene has led to the suggestion that p38 can function as a tumour suppressor. The transforming potential of oncogenes is increased in fibroblasts from these animals as well as their tumorigenic potential in nude mice (Bulavin *et al*., 2002; Brancho *et al*., 2003; Bulavin and Fornace, 2004; Timofeev *et al*., 2005). Suppression of p38 function also plays a critical role in Ras-induced transformation (Ellinger-Ziegelbauer *et al*., 1999; Pruitt *et al*., 2002). The tumour suppressive effects of p38 appear to be mediated in several different ways. p38 is involved in both the activation of p53 and in p53-induced apoptosis and acts as negative regulator of cell cycle progression (Kummer *et al*., 1997; She *et al*., 2001; Bulavin and Fornace, 2004; Bradham and McClay, 2006). p38 is also activated by oncogenic stresses and plays a role in Ras-induced senescence in mouse embryo fibroblasts (Molnar *et al*., 1997; Bulavin *et al*., 2003). Such findings suggest a decrease in p38 activity plays an important role in cancer. In support of this notion, p38 activity has been shown to be reduced in hepatocellular carcinomas in comparison to adjacent normal tissue, with tumour size inversely related to p38 activity (Iyoda *et al*., 2003).

Many chemotherapeutic agents require p38 activity for the induction of apoptosis (Olson and Hallahan,

2004; Bradham and McClay, 2006). Inhibition of p38 activity has been reported to enhance apoptosis in response to DNA-damaging agents such as doxorubicin and cisplatinin as well as microtubule-disrupting agents such as taxol, vicristine and vinblastine (Deacon *et al*., 2003; Losa *et al*., 2003; Lee *et al*., 2006).

MEK4/MKK4

MEK4/MKK4 is a MAPKK for both JNK and p38. It is consistently inactivated by mutation in many cancers including cancers of the pancreas, bile ducts, breast, colon, lung and testis, however at a low frequency around 5% (Cunningham *et al*., 2006). In serous ovarian cancer MEK4 expression was downregulated in 75% of cases (Nakayama *et al*., 2006). There is also evidence that MEK4 suppresses metastasis based on its down-regulation in prostate and ovarian cancers with a high risk of metastasis (Kim *et al*., 2001; Yamada *et al*., 2002). The mechanism how MEK4 antagonizes tumorigenesis and metastasis is currently unknown. Experiments where highly metastatic AT6.1 prostate cancer cells were subjected to individual stresses and tested for colony formation showed that MEK4 expression only had a clear inhibitory effect when at least three stress factors, deprivation of anchorage, growth factor starvation and low pH were combined (Robinson *et al*., 2003). Surprisingly, human cancer cells where the MEK4 gene was knocked out proliferated similar to their parental cells counterparts *in vitro*, but produced fewer metastases when inoculated into mice (Cunningham *et al*., 2006). This is contrary to what one would expect from tumour suppressor gene. However, taken together these results suggest that stress pathways actually may be blind arbitrators that can support tumorigenesis by protecting cells against stress connected with malignant transformation, but also can initiate apoptosis if stress levels exceed a threshold and then act as tumour suppressors.

Outlook

The role of MAPKs in cancer is as pleiotropic as cancer itself. Often we are presented with contradictory findings, which we cannot explain. However, on occasions where such discrepancies could be resolved it usually turned out that they were two sides of the same coin. Most mechanisms can protect or harm depending on the context and strength of activation. The immune system is a prime example. Although our survival is dependent on the ability to vigorously respond to infections with pathogens, similar mechanisms cause allergies or deleterious autoimmune diseases. As much as cancer can be perceived as a disease resulting from faulty inter- and intracellular communications, it also may be perceived as a disease using unusual forms of communication or communication in an unusual form. In either case there should be underlying rules, which we have yet learn to decipher in order to talk cancer cells into resigning.

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