Molecular Pathways: Mitogen-Activated Protein Kinase Pathway Mutations and Drug Resistance

Antonia L. Pritchard and Nicholas K. Hayward

Abstract

Receptor tyrosine kinases are a diverse family of transmembrane proteins that can activate multiple pathways upon ligation of the receptor, one of which is the series of mitogen-activated protein kinase (MAPK) signaling cascades. The MAPK pathways play critical roles in a wide variety of cancer types, from hematologic malignancies to solid tumors. Aberrations include altered expression levels and activation states of pathway components, which can sometimes be attributable to mutations in individual members. The V600E mutation of BRAF was initially described in 2002 and has been found at particularly high frequency in melanoma and certain subtypes of colorectal cancer. In the relatively short time since this discovery, a family of drugs has been developed that specifically target this mutated BRAF isoform, which, after results from phase I/II and III clinical trials, was granted U.S. Food and Drug Administration approval in August 2011. Although these drugs produce clinically meaningful increases in progression-free and overall survival, due to acquired resistance they have not improved mortality rates. New drugs targeting other members of the MAPK pathways are in clinical trials or advanced stages of development. It is hoped that combination therapies of these new drugs in conjunction with BRAF inhibitors will counteract the mechanisms of resistance and provide cures. The clinical implementation of next-generation sequencing is leading to a greater understanding of the genetic architecture of tumors, along with acquired mechanisms of drug resistance, which will guide the development of tumor-specific inhibitors and combination therapies in the future. Clin Cancer Res; 19(9); 2301–9. ©2013 AACR.

Background

Cells respond to certain growth factors, cytokines, and hormones via activation of receptor tyrosine kinases (RTK), with activation of subsequent signaling cascades ultimately altering cellular processes and the expression of genes that encode proteins controlling cellular proliferation, through regulation of the cell cycle, as well as cell death through apoptosis. The RTKs are transmembrane glycoproteins with an intracellular tyrosine kinase domain that activate tiers of effector proteins via an initial phosphorylation event. There are 4 main signaling pathways induced by members of the RTK family, mediated by (i) mitogen-activated protein kinase (MAPK), (ii) the lipid kinase phosphatidylinositol-3-kinase (PI3K), (iii) STAT, and (iv) phospholipase Cβ. The main focus of this review is the pathway mediated via MAPKs, with particular attention to mutations that lead to dysregulation of cancer cell proliferation and to recent advances in pharmaceutical targeting of pathway members for cancer therapy.

The MAPK signaling cascade is highly conserved between different eukaryotic cell types and is composed of 3 to 5 tiers of kinase families (MAP4K, MAP3K, MAP2K, MAPK, and MAPK-activated protein kinases (MAPKAPK; Fig. 1), with one or more of each tier phosphorylating and activating components of the next tier. The canonical MAPK signaling pathway is shared by 4 cascades, which are classified by the MAPK family member at the end of the phosphorylation cascade initiated by the RTK: (i) extracellular signal-related kinases (ERK) 1 and 2, (ii) c-JUN N-terminal kinase (JNK) 1, 2, and 3, (iii) p38-MAPK, and (iv) ERK5 (Fig. 1). Although growth factors are considered to be the main regulators of the ERK1/2 cascade, cellular stress the main inducer of the JNK and p38–MAPK cascades, and either growth factors or stress as activators of the ERK5 cascade. Considerable evidence exists for cross-talk between various components of the pathways, meaning that ultimately the cascades can cooperate to transmit certain signals.

The ERK1/2 Signaling Cascade

Signaling via small G-proteins, for example the RAS family, activates the ERK1/2 signaling cascade. The small G-proteins are recruited by RTKs via SH2/SH3 domain
containing protein intermediates, resulting in active RAS binding to MAP3K RAF family members (Fig. 1). Upon activation, RAF subsequently phosphorylates and thus activates MAP2K 1 and 2 (also known as MEK1/2), which thereafter act as dual specificity kinases, phosphorylating Tyr and Thr residues of the MAPK ERK1/2 proteins (Fig. 1).

Importantly, thus far, the ERK1/2 proteins are the only identified targets of MAP2K1/2, indicating that there is no

Growth factors, 

Inflammatory cytokines, FASL

Extracellular 

Intracellular 

MAP3K 

MAP2K 

MAPK 

Targets of MAPK 

Kinases 

Transcription factors

Figure 1. The 4 canonical MAPK signaling pathways mediating the transmission of an external signal down through the cell to effector proteins that alter cellular proliferation and survival. Signaling pathways should not be considered as purely linear, as there is considerable cross-talk, both between the various MAPK cascades and also with the other RTK-stimulated signaling pathways. This cross-talk predominantly occurs between members of the upper tiers of the network, with the lower tiers providing specificity. For simplicity, this figure shows the main linear pathways only. Additional components of the MAPK super-family exist (e.g., MAP4K2, A-RAF, MAP3K6, MAP3K9, MAP3K10, MAP3K13, MAP3K14, MAP4, MAPK6, MAPKAPK2, and serine/threonine (Ser/Thr) kinase effector proteins, such as MNK1, MNK2, MSK1, MSK2, RPS6KB1, and RPS6KB2), but as many have not been assigned to specific pathways they have not been included in this figure, to aid overall interpretation of the pathways. Similarly, because there is tissue-specific expression of some members of the PAK family, all of them have not been included in the figure. This figure was based on pathway information available from BioCarta (www.biocarta.com), in accordance with their terms and conditions and with their express permission to use the data in the creation of this diagram.
Mutations within members of the ERK1/2 signaling cascade

Oncogenic mutations of the RAS family of genes encode amino acid alterations at 3 main residues—Gly12, Gly13, and Gln61—each of which prevents the hydrolysis of bound guanosine triphosphate to guanosine diphosphate, resulting in a constitutively active form of the protein. Mutations in members of the RAS family are present in approximately 17% of human cancers [Catalogue of Somatic Mutations in Cancer (COSMIC) database; Table 1]. Constitutive activation of RAS is likely to have knock-on effects on other branches of the MAPK pathway due to cross-talk between the upper tiers of the cascade. Activating mutations have also been described in BRAF in several cancer types, including colorectal cancer (6) and non–small cell lung cancer (7). The mutation frequency is particularly high in melanoma (approximately 45%, compared with 21% of all cancer types; Table 1), with more than 90% of these being mutated at the V600 amino acid residue (7, 8).

The V600 amino acid alteration is not exclusively found in cancers, with premalignant lesions, such as melanocytic nevi, also carrying this mutation (9); the implication is that BRAF mutations alone are not sufficient to drive malignancy. Mutations in RAF1 (also known as CRAF) are less common (approximately 2%, COSMIC database; Table 1); however, many studies have shown increased expression of RAF1 in a wide variety of primary human cancers (10, 11). The paucity of oncogenic mutations in RAF1 has been hypothesized because of 2 sites of phosphorylation being required for activation; thus, the protein would require 2 separate mutational hits to constitutively turn on the kinase.

It is uncommon for tumors to contain both RAS and RAF mutations, suggesting that activation of just one member of the cascade is all that is required for oncogenesis.

The JNK1/2/3 Signaling Cascade

The JNK branch of the MAPK pathway is primarily activated in response to stress stimuli, such as UV radiation, DNA damage, and inflammatory cytokines; however, it can also be weakly activated by growth factors and cytokines. These stimuli either directly activate the G-protein RAC1, or alternatively activate the RAS family, which in turn also recruits and phosphorylates RAC1, which then acts via the p21-protein activated kinase (PAK) family to phosphorylate members of the MAP3K family. At least 14 members of the MAP3K family have been linked to activation of the JNK signaling cascade, for example, MAP3K4 and MAP3K12 (Fig. 1). The MAP3K tier dual-phosphorylates MAP2K4 (Mkk4) at Ser271 and Thr275, which in turn activate MAPK8 (JNK), MAPK9 (JNK2), and MAPK10 (JNK3). JNK and JNK2 are ubiquitously expressed in humans, whereas JNK3 is primarily found in the brain, heart, and testis. A wide variety of cellular processes are activated by the JNK pathway, including apoptosis (12), signaling molecules (13), and transcription factors, such as JUN (14), JUND (15), and TP53 (16).

Mutations in the JNK signaling pathway

Inactivating mutations in MAP2K4 have led to its classification as a tumor suppressor gene (17); deletion and epigenetic silencing of this gene have also been reported in breast (18), biliary (18), pancreatic (18), and prostate (19) cancers. Although the main targets of MAP2K4 are the JNK family, it also has a degree of cross-talk with members of the p38 branch of MAPK signaling (20). As JNK has been linked to apoptosis, these inactivating events have been hypothesized to disrupt the signals intended to trigger cell death. In addition, RAS mutations may directly interact with JNK, bypassing the usual pathway through RAC1 (ref. 21; Fig. 1). Recently, an activating RAC1 mutation has been found at high frequency in melanoma (22, 23).

The p38–MAPK Signaling Cascade

The p38–MAPKs are strongly activated by environmental stresses and inflammatory cytokines; however, growth factors are able to weakly activate this pathway. p38–MAPKs have been shown to play an important role in cell-cycle checkpoint control at the G0, G1–S, and G2–M transitions in a cell-specific manner. The p38–MAPKs require dual phosphorylation of the Thr-Gly-Tyr motif in the activation loop by MAP2K3 (MEK3) or MAP2K6 (MEK6) to become fully enzymatically active. These members of the MAP2K family are highly specific for the p38–MAPK proteins; however, the MAP3K family members that activate the MAP2K tier are tissue and stimuli specific (Fig. 1). In addition, a role for members of the Rho family and heterotrimeric G-protein–coupled receptors has also been implicated (24, 25). There are 4 members of the p38–MAPK family: MAPK11 (p38–α), MAPK12 (p38–β), MAPK13 (p38–δ), and MAPK14 (p38–γ), which display tissue-specific patterns of expression (26, 27). The 4 subunits have additionally been shown to differentially activate certain target substrates, for example, the microtubule-associated protein tau (28), suggesting specialized functions for each isoform. Downstream targets of members of the p38–MAPK pathway include the transcription factors TP53 (29), STAT1, MEF (30), and NF-xB (31), cytoskeletal proteins, and other kinases (Fig. 1).

Mutations in the p38–MAPK signaling pathway

p38–MAPK cannot be classified as either a tumor suppressor or an oncosgene, as it has roles in preventing cellular proliferation in response to cellular stress causing DNA damage (32, 33), as well as increasing angiogenesis in response to a hypoxic environment caused by tumor mass (34). Mutations in the p38–MAPK signaling pathway are relatively rare in comparison with the other branches of the MAPK signaling network (Table 1). Nevertheless, studies...
Table 1. Mutation frequencies of key molecules within the 4 MAPK canonical pathways reported in the COSMIC\(^a\) or compiled from recently published exome/whole-genome next-generation sequencing data\(^b\)

<table>
<thead>
<tr>
<th>Protein (alternate name)</th>
<th>Main MAPK pathway</th>
<th>Number mutations (total samples analyzed; %)</th>
<th>Mutations in melanoma(^c) (total samples analyzed; %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G-protein/target</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS, NRAS, and HRAS</td>
<td>ERK-1/-2</td>
<td>29,337 (174,571; 16.8%)(^a)</td>
<td>1,014 (7,403; 13.7%)(^a)</td>
</tr>
<tr>
<td>RAC1</td>
<td>JNK</td>
<td>8 (658; 1.2%)(^a)</td>
<td>12 (247; 4.9%)(^b)</td>
</tr>
<tr>
<td>PAK1</td>
<td>JNK</td>
<td>29 (970; 2.9%)(^a)</td>
<td>2 (247; 0.8%)(^b)</td>
</tr>
<tr>
<td>PAK2</td>
<td>JNK</td>
<td>12 (958; 1.3%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>PAK4</td>
<td>JNK</td>
<td>13 (958; 1.4%)(^a)</td>
<td>5 (247; 2.0%)(^a)</td>
</tr>
<tr>
<td><strong>MAP4K tier</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP4K3 (MTK1)</td>
<td>ERK5</td>
<td>44 (579; 7.6%)(^a)</td>
<td>8 (247; 3.2%)(^b)</td>
</tr>
<tr>
<td>MAP4K4 (HGK)</td>
<td>ERK5</td>
<td>42 (578; 7.3%)(^a)</td>
<td>8 (247; 3.2%)(^b)</td>
</tr>
<tr>
<td>MAP4K1 (HPK)</td>
<td>p38</td>
<td>44 (1,003; 4.4%)(^a)</td>
<td>6 (247; 2.4%)(^b)</td>
</tr>
<tr>
<td>MAP4K5 (KHS)</td>
<td>p38</td>
<td>15 (553; 2.7%)(^a)</td>
<td>2 (247; 0.8%)(^b)</td>
</tr>
<tr>
<td><strong>MAP3K tier</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-RAF</td>
<td>ERK-1/-2</td>
<td>27,391 (131,564; 20.8%)(^a)</td>
<td>5,084 (11,273; 45%)(^a)</td>
</tr>
<tr>
<td>RAF-1 (C-RAF)</td>
<td>ERK-1/-2</td>
<td>32 (1,510; 2.1%)(^a)</td>
<td>3 (247; 1.2%)(^b)</td>
</tr>
<tr>
<td>MAP3K4 (MTK1)</td>
<td>JNK</td>
<td>77 (600; 12.8%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>MAP3K12 (DLK)</td>
<td>JNK</td>
<td>37 (620; 5.9%)(^a)</td>
<td>7 (247; 2.8%)(^a)</td>
</tr>
<tr>
<td>MAP3K2 (MEKK2)</td>
<td>ERK5</td>
<td>7 (1,394; 0.5%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>MAP3K3 (MEKK3)</td>
<td>ERK5</td>
<td>28 (993; 2.8%)(^a)</td>
<td>2 (247; 0.8%)(^a)</td>
</tr>
<tr>
<td>MAP3K8 (MEKK1)</td>
<td>ERK5</td>
<td>23 (991; 2.3%)(^a)</td>
<td>4 (247; 1.6%)(^a)</td>
</tr>
<tr>
<td>MAP3K5 (ASK1)</td>
<td>p38</td>
<td>58 (1,030; 5.6%)(^a)</td>
<td>27 (247; 10.9%)(^b)</td>
</tr>
<tr>
<td>MAP3K7 (TAK1)</td>
<td>p38</td>
<td>28 (993; 2.8%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>MAP3K11 (PTK1)</td>
<td>p38</td>
<td>24 (990; 2.4%)(^a)</td>
<td>9 (247; 3.6%)(^b)</td>
</tr>
<tr>
<td><strong>MAP2K tier</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP2K1 (MEK1)</td>
<td>ERK-1/-2</td>
<td>52 (1,468; 3.5%)(^a)</td>
<td>7 (247; 2.8%)(^a)</td>
</tr>
<tr>
<td>MAP2K2 (MEK2)</td>
<td>ERK-1/-2</td>
<td>13 (516; 2.5%)(^a)</td>
<td>5 (247; 2.0%)(^b)</td>
</tr>
<tr>
<td>MAP2K7 (MKK7)</td>
<td>JNK</td>
<td>19 (1,449; 1.3%)(^a)</td>
<td>0 (247; 0%)(^b)</td>
</tr>
<tr>
<td>MAP2K4 (MEK4)</td>
<td>JNK</td>
<td>100 (3,658; 2.7%)(^a)</td>
<td>4 (247; 1.6%)(^a)</td>
</tr>
<tr>
<td>MAP2K5 (MEK5)</td>
<td>ERK5</td>
<td>16 (555; 2.9%)(^a)</td>
<td>5 (247; 2.0%)(^b)</td>
</tr>
<tr>
<td>MAP2K3 (MEK3)</td>
<td>p38</td>
<td>0 (781; 0%)(^a)</td>
<td>11 (247; 4.5%)(^b)</td>
</tr>
<tr>
<td>MAP2K6 (MEK6)</td>
<td>p38</td>
<td>20 (1,420; 1.4%)(^a)</td>
<td>5 (247; 2.0%)(^b)</td>
</tr>
<tr>
<td><strong>MAPK tier</strong></td>
<td></td>
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</tr>
<tr>
<td>MAPK3 (ERK1)</td>
<td>ERK-1/-2</td>
<td>10 (1,417; 0.7%)(^a)</td>
<td>0 (247; 0%)(^b)</td>
</tr>
<tr>
<td>MAPK1 (ERK2)</td>
<td>ERK-1/-2</td>
<td>17 (1,413; 1.2%)(^a)</td>
<td>4 (247; 1.6%)(^b)</td>
</tr>
<tr>
<td>MAPK8 (JNK)</td>
<td>JNK</td>
<td>34 (1,608; 2.1%)(^a)</td>
<td>5 (247; 2.0%)(^a)</td>
</tr>
<tr>
<td>MAPK9 (JNK2)</td>
<td>JNK</td>
<td>26 (1,414; 1.8%)(^a)</td>
<td>0 (247; 0%)(^b)</td>
</tr>
<tr>
<td>MAPK10 (JNK3)</td>
<td>JNK</td>
<td>3 (1,393; 0.21%)(^a)</td>
<td>6 (247; 2.4%)(^a)</td>
</tr>
<tr>
<td>MAPK7 (ERK5)</td>
<td>ERK5</td>
<td>30 (1,414; 2.1%)(^a)</td>
<td>4 (247; 1.6%)(^a)</td>
</tr>
<tr>
<td>MAPK11 (p38-d)</td>
<td>p38</td>
<td>6 (1,398; 0.4%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>MAPK12 (p38-i)</td>
<td>p38</td>
<td>8 (1,404; 0.6%)(^a)</td>
<td>0 (247; 0%)(^b)</td>
</tr>
<tr>
<td>MAPK13 (p38-i)</td>
<td>p38</td>
<td>12 (1,609; 0.7%)(^a)</td>
<td>2 (247; 0.8%)(^b)</td>
</tr>
<tr>
<td>MAPK14 (p38-a)</td>
<td>p38</td>
<td>16 (1,446; 1.1%)(^a)</td>
<td>5 (247; 2.0%)(^b)</td>
</tr>
<tr>
<td><strong>MAPKAPK tier</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSK1 (RPS6KA1)</td>
<td>ERK-1/-2</td>
<td>20 (978; 2%)(^a)</td>
<td>4 (247; 1.6%)(^a)</td>
</tr>
<tr>
<td>RSK2 (RPS6KA3)</td>
<td>ERK-1/-2</td>
<td>41 (1,515; 2.7%)(^a)</td>
<td>0 (247; 0%)(^b)</td>
</tr>
<tr>
<td>RSK3 (RPS6KA2)</td>
<td>ERK-1/-2</td>
<td>45 (1,618; 2.8%)(^a)</td>
<td>8 (247; 3.2%)(^b)</td>
</tr>
<tr>
<td>MSK1 (RPS6KA5)</td>
<td>p38</td>
<td>48 (1,445; 3.3%)(^a)</td>
<td>5 (247; 2.0%)(^a)</td>
</tr>
<tr>
<td>MAPKAPK5 (PRAK)</td>
<td>p38</td>
<td>13 (1,214; 1.1%)(^a)</td>
<td>1 (247; 0.4%)(^b)</td>
</tr>
<tr>
<td>MAPKAPK3 (3PK)</td>
<td>p38</td>
<td>2 (1,601; 0.1%)(^a)</td>
<td>2 (247; 0.8%)(^b)</td>
</tr>
</tbody>
</table>

\(^a\)COSMIC (94, 95).
\(^b\)Compiled from refs. 22, 23, 96–100.
\(^c\)Malignant melanoma: includes all subtissues and all subhistology types, including areas of low UV exposure, mucosal tissues, and those with high UV exposure.
have shown p38–MAPK dysregulation in hematologic malignancies (35, 36) and breast (37), prostate (38), gastric (39), and lung (40) cancers.

The ERK5 Signaling Cascade

The ERK5 signal cascade is stimulated by a variety of extracellular stimuli, such as growth factors (41, 42), inflammatory cytokines (43), and types of cellular stress, such as hypoxia (44) and laminar shear stress (45). These signals lead to a variety of cellular responses, including angiogenesis, antiapoptosis, cell differentiation, and cell proliferation (refs. 46–48; Fig. 1). These external stimuli activate the MAP3K tier, including MAP3K2 and MAP3K3, which specifically phosphorylate the Ser311/Thr315 residues of MAP2K5 (MEK5; ref. 49); MAP2K5 is the only known protein able to activate ERK5 (Fig. 1). To fully activate ERK5, MAP2K5 must dual-phosphorylate the Thr218 and Tyr220 residues of ERK5, which then results in further phosphorylation of MAP2K5 as well as autophosphorylation of ERK5, resulting in maximal activity (50, 51).

Mutations in the ERK5 signaling cascade

Oncogenically mutated RAS has been reported to activate the ERK5 pathway in certain cell types (52); however, this finding has not been confirmed by other studies (53). The wild-type forms of RAS do not seem to stimulate this cascade (54). Mutations in the ERK5 pathway mainly occur at the MAP4K level, with only a low proportion of mutations reported in other tiers of the cascade (Table 1). Nonetheless, ERK5 is overexpressed in several endocrine-related cancer types (55, 56), suggesting a role for this pathway during the malignant process. Evidence suggests this is mediated, at least in part, via dysregulated miR-143 in prostate cancer cells (57) and via an overexpression of STAT3 in breast cancer cells (58). Whether there is a link between these 2 observations is yet to be elucidated.

Clinical–Translational Advances

Given the important roles played by the MAPK pathway in cancer development and progression, intense focus has been directed toward therapeutically targeting these dysregulated signals. Although the RAS protein is mutated in a high proportion of cancers and has an impact on a larger number of signaling cascades, a successful strategy to pharmacologically target it is yet to be discovered (59). In contrast, other members of the MAPK network downstream of RAS have proved highly tractable to pharmacologic inhibition. Successfully developed therapies have included small-molecule nonATP competitive allosteric MEK inhibitors (MEKi), for example, BAY 43-9006 (sorafenib; ref. 60) and trametinib (61) and inhibitors of RAF family members, including vemurafenib and dabrafenib (62, 63). The BRAF inhibitors (BRAFi) represent a particularly novel and innovative direction for emerging cancer therapies, as they specifically target the V600E/K-mutated isoform of the protein. These BRAFi have been subject to intense research interest, and recent reviews succinctly summarize this wealth of data (64, 65). Paradoxically, the BRAFi are capable of accelerating growth in tumors (wild type) at the V600E mutation location in BRAF (66), which emphasizes the importance of somatic mutation screening for identification of patients most suitable for receiving these therapies.

Results from clinical trials on MAPK pathway inhibition

In phase I/II trials of the BRAFi, vemurafenib has shown good response rates in the majority of patients with melanoma (67, 68). Moreover, in a phase III trial, vemurafenib increased progression-free and overall survival, although not mortality rate, in patients with BRAF V600E mutation–positive melanomas compared with those treated with dacarbazine (62). Initial trials excluded patients with brain metastasis; however, a recently published phase II trial of dabrafenib revealed that approximately 30% of patients achieved an overall cranial response in brain metastasis of primary melanoma (69). Stage II trials of vemurafenib for patients with brain metastases are currently recruiting (Clinical trial identifier; NCT01378975).

Further encouraging results of targeting the MAPK pathway in melanoma come from a phase III trial of the MEKi trametinib, which showed significantly improved progression-free and overall survival compared with patients treated with either dacarbazine or paclitaxel (70). In subsequent phase I and II trials, combined therapy of trametinib with a BRAFi (dabrafenib) showed significantly better response rates and progression-free survival than either monotherapy (63).

In addition, targeted agents against activated KIT, an infrequently mutated RTK in melanoma, have also shown promise in some patients (71–73), although results from large phase III randomized clinical trials of KIT inhibitors are keenly awaited (e.g., NCT01280565).

Despite the initial optimism following the introduction of molecularly targeted therapies in the clinic, considerable reassessment has been needed, as the vast majority of patients developed resistance to the drugs. Fortunately, in the case of BRAFi, therapeutic resistance was identified early and the research field moved quickly to identify potential causes.

Mechanisms of Resistance to BRAF Inhibitors

In vivo

Investigations comparing tumors from patients with melanoma pre- and postdevelopment of BRAFi resistance have uncovered a multitude of mechanisms leading to regrowth/recurrence of metastatic melanoma in individuals treated with such targeted therapies. These include increased expression and signaling through various RTKs, such as platelet-derived growth factor receptor-β (74, 75) or insulin-like growth factor I receptor (76), upregulation of the kinase MAP3K8/COT (77), amplification of the BRAF gene resulting in higher levels of expression (78), alternative splicing of BRAF leading to dimerization (79), NRAS mutation (74, 79, 80), and mutation of MEK (81), although there is evidence that the latter does not always lead to
development of BRAFi resistance (75). Interestingly, treatment with vemurafenib did not result in meaningful clinical outcomes when in the 8% to 10% of colon cancers that carry BRAF V600E mutations. The cause of this was identified as being feedback reactivation of the MAPK pathway via the RTK EGFR receptor (EGFR; ref. 82), implying that these tumors have innate, rather than acquired resistance to BRAFi. This resistance mechanism does not seem to be operative in melanoma, which has only very low levels of EGFR expression (82). Importantly, each of the resistance pathways in melanoma has the same endpoint—reactivation of the ERK1/2 branch of the MAPK cascade. Hence, high levels of MAPK activity seem central to, or essential for, melanoma growth in vivo.

**In vitro**

A number of studies have addressed mechanisms of BRAFi resistance in vitro by generating subclones of melanoma cell lines that develop resistance to RAF inhibitors through prolonged culture and selection with these agents. Like the diverse mechanisms of resistance that develop in patients with melanoma trials in vivo, these also have the common theme of reactivating MAPK. They include upregulation of RAF1 (83) or upstream RTKs such as fibroblast growth factor receptor 3 (84), as well as mutation of NRAS (85) or KRAS (86).

**Stromal mediated**

Whereas all of the mechanisms of BRAFi resistance mentioned above are cell autonomous, Straussman and colleagues showed an alternative paracrine resistance mechanism (87). They showed that fibroblast secretion of hepatocyte growth factor (HGF) activates the RTK MET on adjacent melanoma cells, leading to reactivation of MAPK and resistance to the RAF inhibitor PLX4720. Recombinant HGF was sufficient to induce BRAFi resistance in melanoma cell lines and either HGF neutralizing antibodies or a MET inhibitor blocked PLX4720 resistance (87). In vivo, this association was supported by a correlation with the level of stromal cell HGF expression in tumors of BRAF mutation–positive melanoma patients who developed resistance to BRAFi (87). An important implication of this finding is that dual inhibition of BRAF and HGF or MET could prevent the development of BRAFi resistance in some patients. Thus, clinical trials assessing the efficacy of these combined agents seem warranted.

**Future Developments: Peering Over the Horizon**

The early promise but subsequent failure of single-agent targeted therapies to significantly improve mortality rates in melanoma has led to a somewhat obvious paradigm shift in treatment approaches. Combination therapies are now becoming de rigueur. Given the strong (and potentially necessary) involvement of MAPK signaling in melanoma growth and the development of resistance to BRAFi, it is likely that dual use of a BRAFi with an inhibitor of MEK or MAPK will become standard for treating patients with BRAF mutation–positive melanomas. However, this is also likely to be only the backbone of new combination treatments, with additional agents targeting components of other pathways (e.g., PI3K, PAK, RAC1, or CDK4), or the immune system (see below), being included. Interestingly, cell lines with NRAS or KRAS mutations have shown more sensitivity to IPA3, a small-molecule inhibitor of PAK1, and subcytotoxic doses of IPA3 make cells more sensitive to MEKi (88), thus offering evidence that combined therapies might have synergistic effects and providing possible novel mechanisms by which existing drugs may still yield clinically meaningful outcomes.

It is highly likely that other mechanisms of resistance exist than those discussed above. Future studies will undoubtedly also investigate whether epigenetic changes, which can control activation or silencing of gene expression, occur in cells after BRAFi exposure. Given the recent data from Yancovitz and colleagues showing that clonality of the BRAF V600E mutation occurs both intra- and intertumorally from the same patient (89), it is also highly likely that clonal selection will play a role in resistance to BRAFi.

The advent of the whole-genome sequencing era of genetic research has resulted in high-volume screening of melanoma cell lines and tumors for somatic mutations, which has revealed that it is highly unlikely that driver mutations of a similar frequency to those described in BRAF or RAS family members (Table 1) have yet to be uncovered in other members of the MAPK pathway. It remains to be investigated whether the lower-frequency mutations could have collective effects, due to the considerable degree of crosstalk between branches of the MAPK pathways. Further investigations are clearly required to elucidate the effects of combinations of less common mutations, which in turn could point to new avenues for pharmaceutical intervention. It also remains to be determined whether endpoints other than the dysregulation of MAPK play a vital role in melanoma tumorigenesis; for example, GNAQ and GNA11 are frequently mutated in ocular melanoma and feed into other pathways in addition to the MAPK cascade. If other tumorigenic mechanisms do exist, they might also be a source of resistance to BRAFi.

An alternative to targeting components of the MAPK (and/or other) signaling pathway in cancer treatment is the use of a new wave of immune modulators, such as antibodies to CTLA4 (90), PD1 (91), or PD1L (91), which inhibit negative regulation of cytotoxic CD8+ T cells mediated by these ligands/receptors. For example, in a phase III trial, an anti-CTLA4 antibody (ipilimumab) increased overall survival in patients with melanoma compared with those treated with the glycoprotein-100 (gp100) melanoma antigen immunostimulatory peptide vaccine; combined treatment with ipilimumab and gp100 did not result in improved survival compared with ipilimumab alone (90). In another trial, patients with melanoma treated with a combination of ipilimumab and dacarbazine showed significantly improved overall survival compared with dacarbazine alone (92).
Results from an ongoing combination trial of BRAFi plus ipilimumab (NCT01400451) are eagerly awaited.

Conclusions

In summary, the MAPK pathways activated by RTK ligation have been proved to play an important role in tumorigenesis, as they control cell cycle and proliferation as well as apoptosis, which, when dysregulated, drives abnormal cellular responses. In 2002, the V600E mutation in BRAF was discovered at high frequency in melanoma (7) and to a lower degree in other cancer types (Table 1). In the intervening years, novel therapies were developed that targeted this mutated isoform of BRAF, as rapidly as 2 years following the initial report of V600E mutations (93). These therapies have since been refined, resulting in U.S. Food and Drug Administration approval for vemurafenib being granted in August 2011, with BRAFi being clinically available as a therapeutic option for metastatic melanoma positive for V600E mutations. Although BRAFi do not improve mortality rates, they do result in clinically meaningful increases in progression-free and overall survival. The speed at which the laboratory findings were translated into a meaningful clinical outcome bodes well for future driver mutations identified in the wake of the recent technologic advances allowing rapid and ever more consistent whole-genome sequencing. It is our belief that the first vital steps have been taken toward future treatment regimens in which personalized medicine based on tumor mutation status in combination with standard chemo- and/or immune therapies will become a regular therapeutic course.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

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