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# MEK in cancer and cancer therapy

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ABSTRACT

The mitogen-activated extracellular signal-regulated kinase (MEK) pathway is one of the best-characterized kinase cascades in cancer cell biology. It is triggered by either growth factors or activating mutations of major oncogenic proteins in this pathway, the most common being Ras and Raf. Deregulation of this pathway is frequently observed and plays a central role in the carcinogenesis and maintenance of several cancers, including melanoma, pancreatic, lung, colorectal, and breast cancers. Targeting these kinases offers promise of novel therapies. MEK inhibitors (MEKi) are currently under evaluation in clinical trials and many have shown activity. In this review, we comprehensively examine the role of the MEK pathway in carcinogenesis and its therapeutic potential in cancer patients, with a focus on MEKi. We describe the clinical perspectives of MEKi in the two main models of Ras–ERK driven tumors, *BRAF*-mutant ("addicted" to the pathway) and *KRAS*-mutant (non-"addicted"). We also highlight the known mechanisms of resistance to MEKi and emerging strategies to overcome it.



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## 1. Introduction

During the last 30 years, improved understanding of the molecular biology of cancer cells has led to a therapeutic revolution of targeted therapy, with monoclonal antibodies or small molecule inhibitors directed against proteins (mainly kinases) that are specifically over-expressed or mutated in cancer cells. These agents are widely expected to be both more specific to cancer cells and less toxic than cytotoxic agents.

The Ras–Raf–MEK–ERK signaling pathway (Ras–ERK pathway) is one of the best-characterized kinase cascades in cancer cell biology

*Abbreviations:* CRC, colorectal cancer; EMT, epithelial-to-mesenchymal transition; ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; HCC, hepatocellular carcinoma; HR, hazard ratio; MAPK, MAP kinase; MEK, mitogen-activated extracellular signal-regulated kinase; MEKi, MEK inhibitor; NSCLC, non-small cell lung carcinoma; PAC, pancreatic adenocarcinoma; PanIN, pancreatic intraepithelial neoplasia; PFS, progression-free survival; pRb, retinoblastoma protein; TKR, tyrosine kinase receptor.

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(McCubrey et al., 2007). It is triggered by growth factors or activating mutations of oncogenic kinases involved in this pathway. Deregulation of the Ras–ERK pathway is observed in several cancers and yields multiple changes in the expression of numerous genes involved in tumor cell differentiation, proliferation, survival, migration, and angiogenesis (McCubrey et al., 2007). Kinases of this pathway are promising targets for identifying novel therapies.

With oncogenic Ras proving insensitive to currently-available drugs, several inhibitors of downstream kinases, including Raf and mitogen-activated extracellular signal-regulated kinase (MEK), have been developed. Several of these agents are currently under evaluation in clinical trials and a number of them have shown evidence of activity. We comprehensively examine the role of the Ras–ERK pathway in carcinogenesis and as a novel therapeutic oncogenic target. Particular attention is paid to MEK inhibitors (MEKi) as promising therapeutic agents. We describe the clinical perspectives of MEKi in models of Ras–ERK "addicted" tumors (i.e. *BRAF*-mutant) and *KRAS*-mutant "non-addicted" tumor and highlight the role of MEKi in B-Raf inhibitor resistance. Current knowledge of MEKi resistance is detailed and combination strategies to delay or overcome MEKi resistance are evoked.

## 2. The Ras-extracellular signal-regulated kinase pathway

### *2.1. Description of the Ras–extracellular signal-regulated kinase signaling pathway*

The mitogen-activated protein kinase (MAPK) cascades are evolutionarily conserved signal transduction pathways. They are activated in response to extracellular stimuli (growth factors, cytokines, stress) and can mediate both physiological and pathological responses in mammalian cells and tissues. The ERK1/2 pathway (namely, the Ras–ERK pathway) is the best characterized of the MAPK pathways (Dhillon et al., 2007).

The Ras–ERK signaling cascade has been extensively described (McCubrey et al., 2007). Classical activation results from growth factor binding to a tyrosine kinase receptor (TKR), such as EGFR, which triggers receptor dimerization, activation, and transphosphorylation (McCubrey et al., 2007). Adaptor proteins associate with the receptor phosphorylated intracellular domains and recruit guanine nucleotide exchange factors (GEFs) to the cell membrane which activate Ras small GTPases (H-Ras, N-Ras, K-Ras) (McCubrey et al., 2007). Ras proteins have intrinsically low GTPase activity and function as a GDP/GTP-regulated switch, the GTP-bound form being active and the GDP-bound one being inactive (Rajalingam et al., 2007). GEFs promote formation of Ras-GTP by catalyzing the replacement of GDP with GTP, whereas GTPase-activating proteins (GAPs) stimulate GTP hydrolysis to GDP. Activated Ras binds to a spectrum of downstream effector targets, including Raf, and the PI3K–AKT–mTOR and RalGEF–Ral cascades (Rajalingam et al., 2007; Chappell et al., 2011; Neel et al., 2011). Following Ras activation, Raf serine/threonine kinases (A-Raf, B-Raf, and C-Raf) are recruited to the cell membrane and activated in a complex series of events including phosphorylation/dephosphorylation, dimerization, and association with scaffolding complexes (McCubrey et al., 2007). Raf proteins directly activate MEK1 and MEK2 through phosphorylation. B-Raf is a much more potent activator of MEK compared with C-Raf or A-Raf. Other kinases (PAK1 and COT/Tpl2) can also phosphorylate MEKs and modulate their activation (Coles & Shaw, 2002; Ramos, 2008; Johannessen et al., 2010). MEK1 and MEK2 are tyrosine and serine/threonine dual-specificity kinases and subsequently phosphorylate ERK1 and ERK2, resulting in their activation (Ramos, 2008). Of interest, MEKs are very substrate-specific and have no known targets other than ERK proteins. ERK1 and ERK2 are serine/threonine kinases. Upon activation, they activate multiple nuclear and cytoplasmic targets (>600), including transcription factors, kinases, phosphatases and cytoskeletal proteins (McCubrey et al., 2007; Ramos, 2008).

Although conceptually linear, several feedback loops and considerable cross-talk exist between the Ras–ERK pathway and many other signaling cascades (including PI3K–AKT–mTOR, p38 and JNK MAPK, NF-κB, Wnt-β-catenin, Hedgehog, Notch, and TGFβ–SMAD pathways) which may be important for MEKi resistance (Ramos, 2008; De Luca et al., 2012).

### *2.2. Consequences of Ras–extracellular signal-regulated kinase pathway activation in cancer biology*

The Ras–ERK pathway plays a role in all known biological functions acquired during the multistep development of human tumors (Hanahan & Weinberg, 2011) [Fig. 1]. Many of the transcription factors activated by the Ras–ERK pathway are involved in cell proliferation and differentiation (e.g., AP1, c-Myc) (Murphy et al., 2001; McCubrey et al., 2007). In addition, many growth factor genes have binding sites for transcription factors activated by the Ras–ERK pathway, located in their promoter regions. Thus, aberrant activation of this pathway may establish an autocrine/paracrine loop, resulting in self-sufficiency in proliferative signals and continuous stimulation of cell growth (McCubrey et al., 2007; Maurer et al., 2011).

The Ras–ERK pathway alters the expression of many molecules that regulate the cell cycle including senescence markers (p16, p15, and p21), and high levels of ERK signaling can lead to premature cell cycle arrest in the G1 phase (Roovers & Assoian, 2000; Mirza et al., 2004; Gysin et al., 2005; McCubrey et al., 2007). In contrast, this pathway cooperates with PI3K–AKT–mTOR to promote cell cycle progression by inducing the expression of cyclin D1 and repressing $p27^{KIP1}$, activating the CDK4–cyclin D and CDK2–cyclin E complexes and promoting phosphorylation of the retinoblastoma protein (pRb). C-Raf can also phosphorylate pR (Maurer et al., 2011). pRb phosphorylation counteracts its inhibiting effect on E2F1 and increases E2F1-dependent transcriptional activity, leading to entry into the S phase of the cycle.

The Ras–ERK pathway promotes survival by repressing the expression or activity of pro-apoptotic BCL-2 family proteins (BIM, BAD), and by inducing the expression or activation of anti-apoptotic members (BCL-2, BCL-XL, and MCL-1), both of which prevent mitochondrial depolarization (intrinsic death pathway) (Ballif & Blenis, 2001; McCubrey et al., 2007; Balmanno & Cook, 2009). This effect is directly mediated by ERK or through the target kinases RSK and MSK. The Ras–ERK pathway can also inhibit caspase-9 activity by phosphorylation (Allan et al., 2003). Moreover, ERK activation can inhibit apoptosis induced by the death receptors Fas, TRAIL, or TNF (extrinsic death pathway) (Sahu et al., 2011).

In addition, aberrant activation of Ras–ERK pathway activation contributes to senescence evasion by upregulating telomerase. Replicative senescence is caused by telomere shortening after each cell division which ultimately causes a DNA-damaged response mediated by p53 and p21, leading to cell cycle arrest or death. Ets transcription factor, an ERK target, can stimulate the transcriptional activation of the telomerase catalytic subunit gene (hTERT), thereby restoring telomere repeats and supporting the replicative potential of tumor cells (Maurer et al., 2011).

Epithelial-to-mesenchymal transition (EMT) is a process linked to dedifferentiation, by which epithelial cells undergo a phenotypic shift from cells with tight cell–cell junctions, clear basal and apical polarity, and sheet-like growth architecture, expressing epithelial markers such as E-cadherin, into spindle-like fusiform, motile cells which express mesenchymal markers such as vimentin and N-cadherin. It is associated with enhanced cell motility, invasiveness, and resistance to anticancer treatments, and maybe a step toward cancer stem cell genesis. The Ras–ERK pathway is required for EMT and contributes to the maintenance of an undifferentiated/mesenchymal state in tumor cells (Maurer et al., 2011). It cooperates with other pathways (mainly, the TGFβ–SMAD pathway) to upregulate expression of EMT-related genes, including mesenchymal genes and transcription repressors of epithelial