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Differential activity of MEK and ERK inhibitors in BRAF inhibitor resistant melanoma☆

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ABSTRACT

Acquired resistance to BRAF inhibitors often involves MAPK re-activation, yet the MEK inhibitor trametinib showed minimal clinical activity in melanoma patients that had progressed on BRAF-inhibitor therapy. Selective ERK inhibitors have been proposed as alternative salvage therapies. We show that ERK inhibition is more potent than MEK inhibition at suppressing MAPK activity and inhibiting the proliferation of multiple BRAF inhibitor resistant melanoma cell models. Nevertheless, melanoma cells often failed to undergo apoptosis in response to ERK inhibition, because the relief of ERK-dependent negative feedback activated RAS and PI3K signalling. Consequently, the combination of ERK and PI3K/mTOR inhibition was effective at promoting cell death in all resistant melanoma cell models, and was substantially more potent than the MEK/PI3K/mTOR inhibitor combination. Our data indicate that a broader targeting strategy concurrently inhibiting ERK, rather than MEK, and PI3K/mTOR may circumvent BRAF inhibitor resistance, and should be considered during the clinical development of ERK inhibitors.

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1. Introduction

Constitutive signalling through the mitogen activated protein kinase (MAPK) pathway is common in melanoma and often driven by activating mutations in the BRAF kinase (Davies et al., 2002). Potent inhibitors of the $BRAF^{V600}$ mutant protein, dabrafenib and vemurafenib, have produced response rates of 50–60% and improved progression-free and overall survival, compared to dacarbazine, in patients with $BRAF^{V600E}$ mutant metastatic melanoma (Chapman et al., 2011; Falchook et al., 2012b; Flaherty et al., 2010; Hauschild et al., 2012). The use of these targeted therapies is limited, however by the development of drug resistance which occurs in the majority of patients (Falchook et al., 2012b; Flaherty et al., 2010).

Multiple mechanisms of resistance to BRAF inhibition have been identified, and the majority involve MAPK pathway re-activation via alternate *BRAF* transcript splicing, *BRAF* amplification (Poulikakos et al., 2011; Shi et al., 2012), activating mutations in *N-RAS* or *MEK1* (Nazarian et al., 2010; Wagle et al., 2011), over expression of the kinases COT1 and CRAF (Johannessen et al., 2010; Montagut et al., 2008) or activation of receptor tyrosine kinases (RTKs) (Girotti et al., 2013; Shi et al., 2011; Villanueva et al., 2010). Despite the prevalence of persistent MAPK signalling in resistant metastases, inhibition downstream of BRAF using the MEK inhibitor trametinib, as a single agent, had minimal clinical activity in melanoma patients that had progressed on BRAF-inhibitor therapy (Kim et al., 2013). Selective ERK inhibitors have been proposed as alternative salvage therapies in BRAF inhibitor resistant cell models (Hatzivassiliou et al., 2012; Morris et al., 2013).

To examine the potency of ERK inhibition, we compared MEK and ERK inhibitors in nine melanoma cell models with acquired resistance to BRAF inhibition. Resistance in these models was driven by the expression of *BRAF* splice variants, *BRAF* amplification, mutant *N-RAS*, mutant *MEK1* or RTK signalling. Our studies confirm that inhibiting ERK suppressed MAPK signalling and the proliferation of all nine BRAF-inhibitor resistant melanoma models. Nevertheless, ERK inhibition did not induce substantial cell death in five of nine resistant melanoma cell models, and this was often due to the activity and induction of PI3K survival signals. Consequently, the combination of ERK inhibition with PI3K/mTOR inhibition promoted cell death in these BRAF inhibitor resistant melanoma cells, and this combination was more potent than simultaneously co-inhibiting MEK and the PI3K/mTOR cascade. Our data indicate that inhibition of ERK is more effective than inhibiting MEK but both inhibitors fail to consistently induce melanoma cell death. Thus, ERK inhibition alone is unlikely to show sufficient clinical activity in patients with BRAF inhibitor resistant disease. Instead, inhibiting the MAPK pathway at the ERK, rather than MEK node, in combination with PI3K/mTOR inhibition, efficiently and consistently overcomes acquired resistance to BRAF inhibitors.

2. Materials and methods

2.1. Patients, cell culture and compounds

Informed consent was obtained for each patient under approved Human Research Ethics Committee protocols. Melanoma cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Carlsbad, CA) with 10% fetal bovine serum, HEPES and L-glutamine and cultured in a 37 °C incubator with 5% CO_2. Patient derived short-term cultures with acquired resistance to BRAF ± MEK inhibition were generated in the absence of inhibitor (Carlino et al., 2013) and BRAF inhibitor resistant cells derived after chronic drug exposure were maintained in drug. Drug resistance was regularly monitored and confirmed using viability assays (Carlino et al., 2013; Lai et al., 2012). $BRAF^{V600}$ genotype of melanoma cultures was confirmed using PCR-based capillary sequencing (data not shown).

Stocks of dabrafenib (Active Biochem, Maplewood, NJ), trametinib (Selleck Chemicals, Houston, TX), VX-11e (Active Biochem), MEK162 (Selleck Chemicals), SCH772984 (Active Biochem), BEZ235 (Selleck Chemicals), LY294002 (Selleck Chemicals) and RAD001 (Selleck Chemicals), were made in DMSO. Cell authentication was confirmed using the StemElite ID system from Promega (Madison, WI).

2.2. Resistance screen

An RT-PCR resistance screen was used to examine the expression of BRAF splice variants, the complete coding sequence of *MEK1*, *MEK2* and *N-RAS* cDNAs and the 5′ half of the *AKT1* cDNA (encompassing amino acids 1–200) in all BRAF-inhibitor resistant cell lines (Table 1). Reverse transcription reactions were performed using the Superscript III First-Strand Synthesis kit (Life Technologies, Carlsbad, CA) with the oligo dT primer. The *MEK1*, *MEK2*, *N-RAS* and *AKT1* gene products were each amplified from cDNA using Taq polymerase (Fisher Bioteh, Wembley, WA, Australia) and *BRAF* cDNA was amplified with Pfx polymerase (Life Technologies). PCR products were purified using QIAquick PCR purification kit (Qiagen, Limburg, Netherlands) followed by Sanger sequencing on the 3730xl DNA Analyser (AGRF, Westmead, NSW, Australia). Amplification and sequencing primers are listed in Table S1. The identity of mutations was confirmed using an independent RT-PCR product. *B-RAF* relative copy number was determined by quantitative PCR using the Corbett Rotor-Gene 6000 as previously described (Corcoran et al., 2010) (Table 1).

Phosphorylation of RTKs was determined with human phospho-RTK array kits (ART001; R&D Systems, Minneapolis, MN) (Table 1). Two hundred and fifty μg of protein extract was analysed as per the manufacturer's protocol.

2.3. Pharmacological growth inhibition assays

Cultured cells were seeded into 96-well plates (1-$2E^3$ cells per well) and serial dilutions of each inhibitor, prepared in media, were added to cells 24 h after seeding. Cells were incubated for