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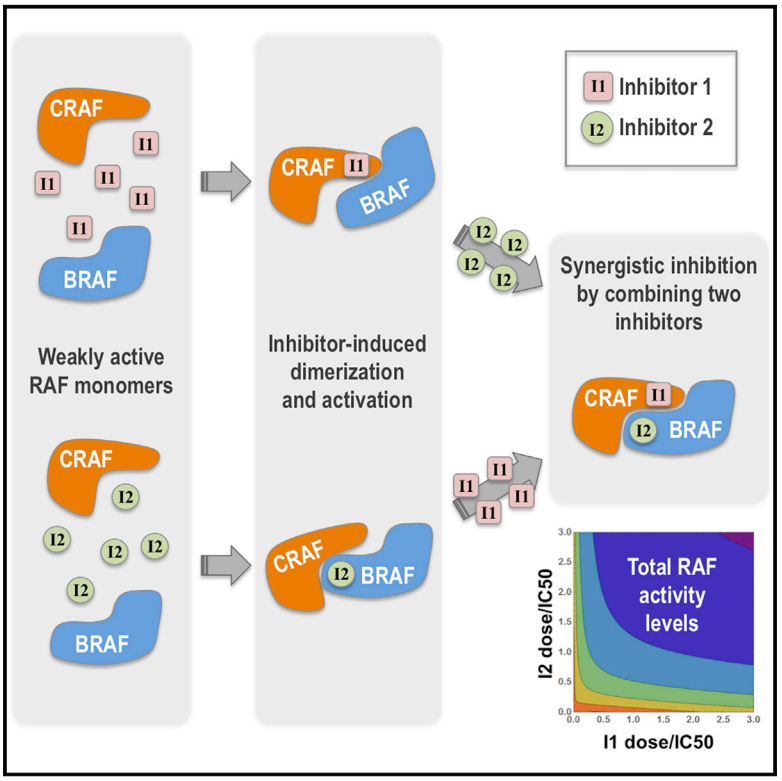
Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects

Graphical Abstract



Highlights

- Allosteric kinase activation caused by dimerization conveys drug resistance
- Thermodynamic factors account for paradoxical kinase activation by a drug
- Accumulation of dimers harboring drug-bound and free protomers drives resistance
- Two inhibitors, ineffective on their own, when combined can abolish drug resistance

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In Brief

Kholodenko has developed a model that describes drug-facilitated dimerization and the emergence of differing drug affinities between free kinase monomers versus dimers. Importantly, the model suggests ways of overcoming drug resistance.

Kholodenko, 2015, Cell Reports *12*, 1939–1949
September 22, 2015 ©2015 The Authors
http://dx.doi.org/10.1016/j.celrep.2015.08.014

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Drug Resistance Resulting from Kinase Dimerization Is Rationalized by Thermodynamic Factors Describing Allosteric Inhibitor Effects

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http://dx.doi.org/10.1016/j.celrep.2015.08.014

SUMMARY

Treatment of cancer patients with ATP-competitive inhibitors of BRAF/CRAF kinases surprisingly increases total kinase activity, especially in wild-type BRAF cells, subverting the desired clinical outcome. Similar inhibition resistance is observed for numerous kinases involving homo/heterodimerization in their activation cycles. Here, I demonstrate that drug resistance resulting from kinase dimerization can be explained using thermodynamic principles. I show that allosteric regulation by inhibitors is described by thermodynamic factors that quantify inhibitor-induced changes in kinase dimerization and the difference in the drug affinity for a free monomer versus a dimer harboring one drug molecule. The analysis extends to kinase homo- and heterodimers, allows for their symmetric and asymmetric conformations, and predicts how thermodynamic factors influence dose-response dependencies. I show how two inhibitors, ineffective on their own, when combined can abolish drug resistance at lower doses than either inhibitor applied alone. Thus, the mechanistic models suggest ways to overcome resistance to kinase inhibitors.

INTRODUCTION

The human genome encodes over 500 protein kinases (Manning et al., 2002). Kinase oncogenic mutations are frequently found in human cancers, many driving the tumor progression and survival of cancer cells (Holderfield et al., 2014; Thomas et al., 2007). Pharmaceutical companies race to add new kinase inhibitors to the ever-increasing number of clinically approved drugs, and protein kinases are currently the second largest targeted protein group following G protein-coupled receptors (Cohen, 2002). Although protein kinase inhibitors often show impressive clinical responses, resistance inevitably occurs. Moreover, many kinase inhibitors have unexpected side effects, surprisingly activating signaling pathways by promoting kinase dimerization (Koppikar et al., 2012; Lito et al., 2013).

Homo- and heterodimerization are key events in the physiological and oncogenic activation of numerous kinases, including receptor tyrosine kinases and multiple cytoplasmic kinases (Bessman et al., 2014; Dey et al., 2005; Hu et al., 2013; Huang et al., 2014; Romano et al., 2014; Wang et al., 2012). In mitogen-activated protein kinase (MAPK) pathways, dimerization is essential for the activation of first-tier kinases (MAP3Ks), including MLK4 and MAP3K11 (Leung and Lassam, 1998). The MAP3Ks of the extracellular signal-regulated kinase (ERK) cascade, BRAF and CRAF (RAF-1, gene name), form homo- and heterodimers as intrinsic steps of their activation cycles (Freeman et al., 2013; Heidorn et al., 2010; Rushworth et al., 2006). The discovery of BRAF mutations, such as BRAF600E (Davies et al., 2002), which lead to oncogenic activation of BRAF and perpetual ERK activation, has made the MAPK pathway a primary target for new cancer drugs. Several BRAF and CRAF inhibitors are undergoing clinical trials or seeing use in the clinic (Rahman et al., 2014). However, nearly all existing RAF inhibitors suppress MAPK signaling only in tumors with mutated BRAF and wild-type RAS. In cells with wild-type BRAF, these inhibitors paradoxically increase the total CRAF/BRAF kinase activity due to inhibitor-induced homo- and heterodimerization of these kinases (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010). RAF inhibitors are also ineffective in cells with constitutively active mutant RAS (Heidorn et al., 2010). Binding to active RAS drives RAF homo- and heterodimerization by inducing RAF conformational changes and bringing two RAF molecules into close vicinity at the plasma membrane (Kholodenko et al., 2000, 2010; Weber et al., 2001). Since RAF heterodimers have significantly higher kinase activity than monomers (Garnett et al., 2005; Lavoie and Therrien, 2015; Rushworth et al., 2006), RAF dimerization is thought to be a major mechanism causing resistance to RAF inhibitors in experiments and clinically (Heidorn et al., 2010; Poulikakos et al., 2011).

Although all the structural details of how existing ATP-competitive inhibitors induce RAF dimerization are not yet worked out, current models suggest that these inhibitors stabilize an active