Fighting Kinase Drug Resistance with Caspase Activators

Jeanne A. Hardy[1,*]
[1]Department of Chemistry, University of Massachusetts, Amherst, MA 01003, USA
*Correspondence: hardy@chem.umass.edu
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Kinase inhibitors are effective cancer therapies. Unfortunately, drug resistance emerges in response to kinase inhibition leading to loss of drug efficacy. In this issue of *Cell Chemical Biology*, Peh et al. (2018) demonstrate that caspase activators effectively delay onset of resistance to kinase inhibitors and are excellent co-therapeutics for a number of tumor types.

Protein kinase inhibitors (PKIs) comprise a highly significant class of anti-cancer treatments. In 2001, imatinib (Gleevec), the first FDA-approved, small-molecule PKI, was approved for the treatment of chronic myelogenous leukemia. Since that hallmark, PKIs have become a standard in the treatment of a number of cancers and development of PKIs has expanded dramatically. Today over 40 PKIs are actively used clinically for indications ranging from cancers of many varieties to rheumatoid arthritis, graph-versus-host disease, and pulmonary fibrosis. Development of this class of inhibitors is still strong, with four FDA approvals so far in 2018. While kinase inhibitors have proven to be extremely effective in decreasing cell proliferation and blocking other pathways required for cancer survival, PKIs are extremely prone to development of drug resistance, which was identified as early as 2001 (Gorre et al., 2001). PKI resistance can emerge within as little as days in cell-based models. In many instances, resistance emerges in human patients within a year of the beginning of treatment (Gillis and McLeod, 2016). Once resistance emerges, patients treated with PKIs become susceptible to reemergence or growth of their cancers. Second generation inhibitors targeting drug resistant kinases have also entered the market (e.g., Debrafenib, Trametinib, Cobimetinib, etc.), but given the cost and time required for the development of these specialized kinase inhibitors, this is not always a feasible long-term solution. In addition, these second generation PKIs are also susceptible to promoting the emergence of drug resistance (Figure 1).

An optimal approach to extending the life of PKIs is the application of new pharmaceuticals that delay or prevent the onset of PKI drug resistance. Peh et al. have developed an approach, co-administration of a procaspase-3 activator PAC-1 with PKIs, which substantially extended the time to development of resistance in treated cells (Peh et al., 2016). PAC-1 is a procaspase-3 activator that functions by removal of zinc from the active site of caspases (Peterson et al., 2009). Caspase-3 is an apoptotic protease that is constitutively inhibited by the presence of physiological levels of zinc (Eron et al., 2018). The zinc-binding properties of PAC-1 enable activation of procaspase-3 to mature caspase-3, and the generation of proteolytic caspase-3 activity.

Caspase-3 plays a leading role in the execution of apoptotic cell death. Because apoptosis leads to the irreversible demise of cells in which it is activated, cancer cells typically have developed multiple overlapping strategies for avoiding apoptosis. If apoptosis can be induced, it is an effective means for clearing cancer cells. Thus PAC-1 has emerged as a potential cancer therapeutic. Upon PAC-1 treatment and during apoptosis, caspase-3 cuts a number of key prosurvival proteins; among these are the kinases MEK1 and MEK2.

A number of approved cancer-targeted PKIs (both first and second generation) function by impacting the mitogen activated protein kinase (MAPK) pathway that signals through RAS/RAF/MEK/ERK (Figure 1). To slow the development of resistance through mutations occurring in RAS, RAF, and BRAF, MEK1/2 inhibitors have been used in combination with PKIs for upstream kinases (Eberlein et al., 2015; Hrustanovic et al., 2015; Tricker et al., 2015). Because MEK1/2 is a major control point in this pathway, dual pressure on the pathway has been shown to be effective at slowing development of drug resistance.

The approach described by the Hergenrother group (Peh et al., 2018) makes use of the same physiological process: imposing dual pressure in the MAPK pathway. Activation of procaspase-3 by PAC-1 leads to caspase-3 cleavage of MEK1/2 (Figure 1), which eliminates the function of MEK1/2. In head-to-head tests, cells were challenged with a PKI alone or in combination with PAC-1 to activate procaspase-3, which cuts and inactivates MEK1/2, or in combination with trametinib, which directly blocks the kinase function of MEK1/2. The authors focused on naive cancer cell lines as well as lines that had already developed resistance to a first generation PKI therapeutic. In particular, this work by Peh et al. (2018) focuses on $BRAF^{V600E}$ (which is targeted by vemurafenib), $EGFR^{T790M}$ (which is targeted by osimertinib), EML4-ALK (which is targeted by ceritinib), or BCR-ABL (which is targeted by imatinib). In all cases, cells treated with PKI alone developed resistance earlier than in combination with PAC-1 or trametinib, underscoring the prior observation that blocking the MEK1/2 control point is an effective co-strategy for preventing emergence of drug resistance. Notably, both return of ERK1/2 phosphorylation, a read out of the MEK1/2 activation state, and the onset of resistance were always later in the presence of PAC-1 than in combination with trametinib.

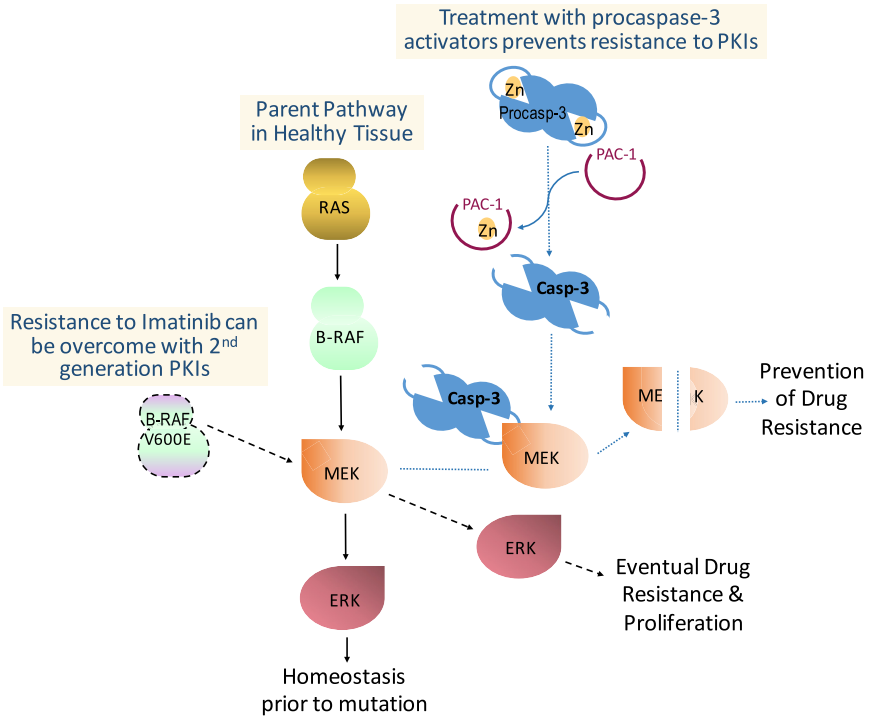

Figure 1. The MAPK (RAS/RAF/MEK/ERK) Pathway

In healthy tissue, the MAPK (RAS/RAF/MEK/ERK) pathway functions normally and prior to mutation supports homeostasis (black arrow pathway). In many cancers, mutations in this pathway drive tumorgenicity and malignancy. The resulting cancers are frequently treated with first generation PKIs. Eventually, drug resistance to the PKIs emerge. A frequent mutation is the B-RAF^{V600E} mutation. Patients hosting the B-RAF^{V600E} mutation can be treated with second generation PKIs that target B-RAF^{V600E}. Unfortunately, drug resistance eventually develops (dashed black arrows). Co-treatment with the procaspase-3 activator PAC-1 (blue finely dashed arrows) leads to degradation of MEK1/2 and prevents development of drug resistance more effectively than currently used regimes.

The authors suggest that the higher efficacy of procaspase-3 activation by PAC-1 at delaying resistance to PKIs is due to the complete removal of MEK1/2 activity that occurs upon proteolysis compared to the reversible inhibition of MEK1/2 by trametinib. Treatment with trametinib or treatment with PAC-1 initially results in decreases in the amount of phosphorylated ERK1/2, indicating that both pathways are effective at blocking the functioning of this pathway. Notably, within 6 hr of treatment with trametinib, ERK1/2 phosphorylation can once again be observed, indicating that inhibition of MEK1/2 is transient. In contrast, activation of procaspase-3 and the resulting degradation of MEK1/2 are substantially more sustained, preventing ERK1/2 phosphorylation for the duration of the experiment. This sustained MEK1/2 and subsequent ERK1/2 inhibition dramatically reduces cells' ability to proliferate and thus form drug-resistant colonies.

In addition to its impact on the MEK pathway, procaspase-3 also plays a more general role in preventing development of drug resistance. Procaspase-3 activation is well known to induce apoptosis. Inducing apoptosis in cancer cells also decreases the likelihood that drug resistance can reemerge. Thus, this mechanism of combatting development of drug resistance holds significant potential over alternative approaches because it impacts two means of the development of drug resistance.

The findings of Peh et al. (2018) suggest that new combination therapies using PAC-1, which eliminate key components of the MAPK pathway, stand to dramatically prolong the utility of kinase inhibiting drugs. The fact that co-administration of procaspase-3 activators (at levels that have been shown to be clinically achievable) worked on four different cell types indicates that this approach may be broadly applicable across cancers that achieve their proliferative state through a number of different mutations in a wide variety of pathways, including the MAPK pathway. Finally, because PAC-1 is currently undergoing human clinical trials (Danciu et al., 2016), combination therapies based on these findings are eminently possible, hopefully in the not-too-distant future.

REFERENCES

Danciu, O.C., Nicholas, M.K., Holdhoff, M., Venepalli, N.K., Hergenrother, P.J., Tarasow, T.M., and Dudek, A.Z. (2016). Phase I study of procaspase activating compound-1 (PAC-1) in the treatment of advanced malignancies. J. Clin. Oncol. *34*, TPS2605.

Eberlein, C.A., Stetson, D., Markovets, A.A., Al-Kadhimi, K.J., Lai, Z., Fisher, P.R., Meador, C.B., Spitzler, P., Ichihara, E., Ross, S.J., et al. (2015). Acquired resistance to the mutant-selective EGFR inhibitor AZD9291 is associated with increased dependence on RAS signaling in preclinical models. Cancer Res. *75*, 2489–2500.

Eron, S.J., MacPherson, D.J., Dagbay, K.B., and Hardy, J.A. (2018). Multiple mechanisms of zinc-mediated inhibition for the apoptotic caspases-3, -6, -7, and -8. ACS Chem. Biol. *13*, 1279–1290.

Gillis, N.K., and McLeod, H.L. (2016). The pharmacogenomics of drug resistance to protein kinase inhibitors. Drug Resist. Updat. *28*, 28–42.

Gorre, M.E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P.N., and Sawyers, C.L. (2001). Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science *293*, 876–880.

Hrustanovic, G., Olivas, V., Pazarentzos, E., Tulpule, A., Asthana, S., Blakely, C.M., Okimoto, R.A., Lin, L., Neel, D.S., Sabnis, A., et al. (2015). RAS-MAPK dependence underlies a rational polytherapy strategy in EML4-ALK-positive lung cancer. Nat. Med. *21*, 1038–1047.

Peh, J., Fan, T.M., Wycislo, K.L., Roth, H.S., and Hergenrother, P.J. (2016). The combination of vemurafenib and procaspase-3 activation is synergistic in mutant BRAF melanomas. Mol. Cancer Ther. *15*, 1859–1869.

Peh, J., Boudreau, M.W., Smith, H.M., and Hergenrother, P.J. (2018). Overcoming resistance to targeted anticancer therapies through small molecule mediated MEK deradation. Cell Chem. Biol. *25*, this issue, 996–1005.

Peterson, Q.P., Goode, D.R., West, D.C., Ramsey, K.N., Lee, J.J., and Hergenrother, P.J. (2009). PAC-1 activates procaspase-3 in vitro through relief of zinc-mediated inhibition. J. Mol. Biol. *388*, 144–158.

Tricker, E.M., Xu, C., Uddin, S., Capelletti, M., Ercan, D., Ogino, A., Pratilas, C.A., Rosen, N., Gray, N.S., Wong, K.K., and Jänne, P.A. (2015). Combined EGFR/MEK inhibition prevents the emergence of resistance in EGFR-mutant lung cancer. Cancer Discov. *5*, 960–971.