In this issue of Clinical Cancer Research, Hoeflich and colleagues (1) report the results of a pharmacogenicomolecular analysis in a large panel of human breast cancer cell lines to identify molecular predictors of responses to a selective small molecule inhibitor of MAP/ERK kinase (MEK). Cell lines and xenografts with a basal-like gene expression signature were sensitive to the small molecule. Consistent with this response, they also exhibited evidence of activation of a RAS-like transcriptional program at baseline. Loss of the tumor suppressor phosphatase PTEN, which results in upregulation of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, was associated with lack of response to the MEK inhibitor despite appropriate inhibition of phosphorylation of MAPK, a downstream substrate of MEK. Interestingly, blockade of MEK resulted in feedback upregulation of the PI3K/Akt pathway but the combined inhibition of both PI3K and MEK synergistically inhibited growth of basal-like breast cancer cells in vitro and in vivo.

Basal-like breast cancers represent a subtype of this disease that has been defined as such because of the resemblance of their gene expression to that of the basal epithelial cell in the normal breast and lack of detectable hormone receptors or amplification of the ErbB2 oncogene. Genomic instability, aberrant DNA repair, and high expression of proliferating genes characterize this breast tumor subtype (reviewed in ref. 2). Loss of PTEN, a lipid phosphatase that negatively regulates the PI3K pathway, was recently reported in approximately 30% of basal-like breast cancers (3). However, the main signaling networks involved in the progression of basal-like breast cancer remain incompletely characterized. Data in this article shed some light on this question. The top 50 genes modulated as a result of expressing gain-of-function versions of HRAS or MEK1 in nontumorigenic MCF10A human mammary epithelial cells were used to do unsupervised hierarchical clustering on a cohort of primary breast tumors. Genes that constitute the RAS/MEK signature from MCF10A cells were preferentially detected in primary breast cancers independently classified as basal-like as well as in basal-like cell lines sensitive to MEK inhibition but not cell lines of a mammary luminal phenotype. Further, this RAS/RAF/MEK transcriptional program was not associated with mutations in RAS or RAF family members or with epidermal growth factor (EGF) receptor signaling. Clearly, additional research is needed to identify networks or kinases that activate MEK in basal-like cancers. Of note, however, the SRC inhibitor dasatinib has shown preferential activity against basal cell lines (4), and phosphorylation of SRC family members has been associated with response to MEK inhibition (5), suggesting SRC family members as potential activators of MEK in mammary tumors of this subtype. In any case, the data of Hoeflich and colleagues suggest the possibility of using a qPCR-based RAS/RAF/MEK activation signature for identifying basal-like breast cancers that are candidates for enrollment into trials with MEK inhibitors.

Loss of the phosphatase PTEN markedly attenuated the response to MEK inhibition in basal-like cancer cells. Cotreatment with PI3K and MEK inhibitors synergistically inhibited cell viability and downregulated cyclin D1. Importantly, MEK inhibition led to feedback upregulation of the PI3K/Akt pathway regardless of the PTEN status in all cell lines examined in this study. These results potentially explain the limited clinical efficacy data with MEK inhibitors and have important implications for their further development as anticancer therapeutics. Despite their ability to inhibit their molecular target in vivo, MEK inhibitors have not shown clinical activity in tumors without RAS or RAF mutations (6). It is thus plausible that in basal-like breast cancers and other tumors in which MEK is activated, both compensatory upregulation of PI3K/AKT and constitutive upregulation of this survival pathway as a result of PTEN loss will limit the antitumor activity of MEK antagonists.
despite appropriate downregulation of MEK activity. Therefore, as long as single agent MEK inhibitors block MEK function as measured by levels of P-MAPK or another reliable MEK effector in tumors, we should not necessarily expect downregulation of cyclin D1 or tumor cell proliferation measured by Ki67 immunohistochemistry or tumor shrinkage. With this knowledge, there should be serious consideration to conduct the clinical development of these drugs mainly if not exclusively in combination with other signaling inhibitors especially in common cancers in which mutations in the RAS/RAF/MEK pathway are not found, such as basal-like breast cancers.

This feedback upregulation of compensatory signaling networks has also been observed with other signal transduction inhibitors such as antagonists of the mTOR kinase. This kinase functions within two multiprotein complexes, TORC1 and TORC2. TORC1 regulates protein translation and is downstream and positively modulated by AKT, whereas TORC2 phosphorylates and activates AKT (reviewed in ref. 7). Inhibition of TORC1 with rapamycin leads to insulin-like growth factor (IGF)-IR/IRS-1-dependent activation of AKT. Inhibition of the IGF-IR with small molecule tyrosine kinase inhibitors prevents rapamycin-induced AKT activation and sensitizes tumor cells to inhibition of TORC1 (8). Furthermore, inhibition of TORC1 with rapalogs in primary breast tumors and in xenografts induces a dose-dependent increase in MAPK activation, which is dependent on an S6K-PI3K-RAS pathway (9). Supporting the notion that this compensation limits the therapeutic inhibition of a single pathway, the combined inhibition of mTOR and MEK has shown synergistic activity against several cancer xenografts (9–11). Thus, although PI3K inhibitors have not yet been shown to induce upregulation of MEK (or an upstream activator of MEK), it is not unreasonable to expect they will do so in cells in which PI3K/AKT inhibition downregulates TORC1 activity downstream.

With the large development of signal transduction inhibitors and their clinical testing in cancer, we should certainly expect to unmask more examples of feedback compensation upon the inhibition of single hubs within signaling networks. For example, inhibition of ErbB2 phosphorylation in ErbB2-overexpressing human breast cancer cells induced by the EGFR tyrosine kinase inhibitor gefitinib is followed by activation of ErbB3 and AKT, thus limiting the inhibitory effect of gefitinib (12). Finally, because activation of mTOR downregulates PDGF receptor signaling (13), it is likely that inhibition of mTOR will also lead to activation of PDGFR in some cancers. In tumors in which this receptor is overexpressed, this response would limit the action of mTOR inhibitors and potentially inform the use of new therapeutic combinations aimed at blocking such compensatory response.

In summary, the elegant pharmacodynamic analysis of Hoeflich and colleagues reminds us of the interconnectedness of the MEK and PI3K pathways in human tumors (Fig. 1). As it applies for basal-like breast cancers, clinical testing of a combination of MEK and PI3K inhibitors is a more rational approach than the testing of each alone. Because gene array profiling is not yet widely clinically available at the time of diagnosis, these investigational trials could enroll patients with breast cancer categorized by routine immunohistochemistry as “triple negative” (for ER, PR, and HER2) using loss of PTEN as a stratification factor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
References


Inhibition of PI3K and MEK: It Is All about Combinations and Biomarkers

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