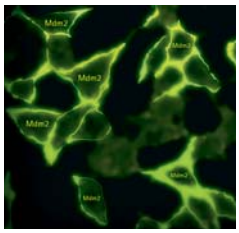
Small-Molecule Inhibitors of Mdm2-MdmX E3 Ligase

- High-throughput auto-ubiquitination assay identified small-molecule inhibitors of the Mdm2-MdmX E3 ligase heterocomplex.
- Mdm2-MdmX ligase inhibitors specifically block ubiquitination of Mdm2 and p53.
- Mdm2 inhibition synergizes with DNA-damaging agents to cause p53-dependent cell death.

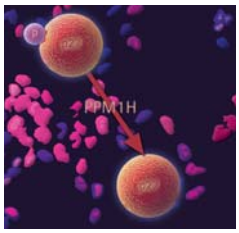


E3 ubiquitin ligases mediate the attachment of ubiquitin to target proteins, marking them for degradation by the proteasome. The murine double minute 2 protein (Mdm2) is an E3 ligase that is a key negative regulator of the p53 tumor suppressor protein and an attractive therapeutic target in cancer. Inhibition of Mdm2 has been shown to reactivate p53 *in vitro* and although various small-molecule inhibitors of Mdm2 have been identified, they tend to be nonspecific or do not directly target the E3 ligase activity. To identify novel inhibitors of Mdm2 E3 ligase activity, Herman and colleagues developed a high-throughput cell-based assay based upon the ability of Mdm2 to auto-ubiquitinate. Two structurally similar compounds, Mdm2 E3 Ligase Inhibitor 23 and 24 (MEL23 and MEL24), were identified and shown to specifically target the E3 ligase activity of the hetero-complex formed by Mdm2 and its homolog MdmX. Treatment of cells with MEL23 and MEL24 inhibited ubiquitination of Mdm2 and p53, increased levels of Mdm2 substrates and p53, and activated transcription of p53 target genes. Importantly, in combination with DNA-damaging agents, MEL23 caused synergistic decreases in cell viability. These data highlight the development of a novel cell-based screen for E3 ligase inhibitors that can be adapted to screen for inhibitors of other E3 ligases. Because the MEL compounds are the first to target the Mdm2-MdmX complex, their discovery forms the basis for future development of a new class of anti-tumor agents. ■

See article, p. 312.

Stabilization of p27 by PPM1H Contributes to Trastuzumab Sensitivity in HER2$^+$ Breast Cancers

- PPM1H is a Ser/Thr phosphatase that protects p27 from proteasomal degradation.
- PPM1H knockdown confers trastuzumab resistance in HER2$^+$ breast cancer cells.
- Low *PPM1H* expression may be associated with worse clinical outcome in patients treated with trastuzumab.

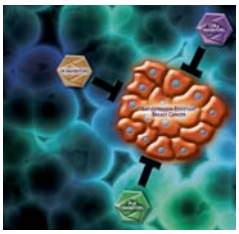


Targeted therapy with the anti-HER2 monoclonal antibody trastuzumab is the major treatment for the 20% of breast cancers with human epidermal growth factor receptor 2 (*HER2*) amplification or overexpression. However, some HER2$^+$ tumors do not respond or acquire resistance to trastuzumab. Lee-Hoeflich and colleagues sought to understand the mechanisms underlying trastuzumab resistance by performing an siRNA screen to identify kinases and phosphatases whose knockdown increased proliferation of HER2$^+$ breast cancer cell lines. The top hit was PPM1H, a poorly characterized member of the PP2C family of Ser/Thr phosphatases. The authors queried known members of the HER2 signaling axis in response to PPM1H knockdown and found that only p27 levels were negatively affected. Because ubiquitin-mediated proteasomal degradation of p27 is initiated by phosphorylation at T187, the authors hypothesized that PPM1H impacts p27 stability via dephosphorylation of p27 at this residue. Indeed, PPM1H specifically dephosphorylates p27 at T187 to prevent its proteasomal degradation, and is therefore likely a key mediator of p27-dependent cell cycle regulation. To determine whether *PPM1H* plays a similar role in mediating the response to trastuzumab therapy *in vivo*, the authors assayed *PPM1H* expression in a panel of 87 HER2$^+$ breast cancer samples from patients who had been treated with trastuzumab. Patients with lower *PPM1H* expression trend toward poor overall survival, suggesting that low *PPM1H* expression may predict which HER2$^+$ breast cancer patients are less likely to respond to trastuzumab therapy. ■

See article, p. 326.

An Estrogen-Independent Role of ER in Endocrine Therapy-Resistant Breast Cancers

- ER promotes cell cycle progression and proliferation of breast cancer cells via estrogen-independent induction of CDK4/E2F targets.
- The estrogen-independent, E2F-induced gene expression profile is a predictor of resistance to estrogen deprivation therapy.
- PI3K inhibition synergizes with ER suppression to induce near-complete regression of ER$^+$ xenografts.

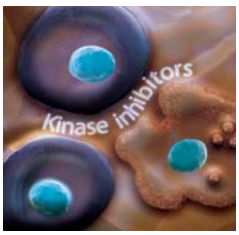


The majority of breast cancers overexpress estrogen receptor α (ER) and are therefore candidates for therapies that inhibit ER-mediated signaling. However, some ER$^+$ breast cancers acquire resistance to therapies that eliminate estrogen production such as aromatase inhibitors. Miller and colleagues identify estrogen-independent, ER-mediated induction of E2F target genes as a key mechanism by which ER$^+$ breast cancer cells escape endocrine therapy. Fulvestrant, a direct ER antagonist, reduces growth of ER$^+$ cells resistant to estrogen depletion, implicating estrogen-independent ER activity in ER$^+$ tumor progression. The authors then identify an E2F target gene expression profile that predicts resistance to multiple forms of estrogen deprivation therapy in ER$^+$ human breast cancers and correlates with high tumor cell proliferation. A complementary kinome siRNA screen revealed that CDK4, which phosphorylates Rb to derepress E2F signaling, is required for hormone-independent growth of ER$^+$ breast cancer cell lines, further demonstrating the importance of the E2F transcriptional program in ER$^+$ tumors and providing a rationale for further study of CDK4 inhibitors. To determine whether estrogen-independent ER activity cooperates with any other pathways implicated in endocrine resistance, the authors honed in on the PI3K pathway, which is hyperactivated in long-term estrogen-deprived cells and has been linked to ER signaling. Combined ER downregulation and PI3K inhibition led to near-complete regression of ER$^+$ xenografts, suggesting that this dual therapy strategy may be useful in treatment of endocrine-resistant tumors. ■

See article, p. 338.

BIM Expression Predicts Kinase Inhibitor Response

- BIM RNA levels predict TKI-induced apoptosis in oncogene-addicted cancers.
- Clinical benefit of TKIs is reduced in patients with low BIM levels.
- Pretreatment levels of BIM RNA can serve as a functional biomarker.



Oncogene-addicted cancers, such as *EGFR*-mutant lung cancers and *HER2*-amplified breast cancers, are dependent upon signaling through the corresponding receptor tyrosine kinase (RTK) to maintain their malignant phenotype. Treatment with tyrosine kinase inhibitors (TKI) often leads to apoptotic cell death, but responses remain heterogeneous with some patients deriving more clinical benefit than others. Recent studies have suggested that the pro-apoptotic Bcl-2 family member BIM plays a critical role in apoptosis induced by targeted therapies. BIM, a BH3-only protein, is primarily bound to anti-apoptotic Bcl-2 family members in the cell, but when freed can activate apoptotic cell death. These observations prompted Faber and colleagues to investigate whether cellular levels of BIM might predict the degree of TKI-induced apoptosis in oncogene-addicted cancers. Using cell line models of *EGFR*-mutant, *HER2*-amplified, *PIK3CA*-mutant, and *BRAF*-mutant cancers, the authors found that pretreatment levels of BIM RNA predicted the degree of apoptotic response induced by the respective TKIs, but not traditional chemotherapies. Induction of BIM expression sensitized low BIM cancers to targeted therapies *in vitro* and knockdown of BIM expression abrogated both the apoptotic response and tumor regression *in vivo*. Importantly, pretreatment tissue samples from lung cancer patients confirmed that the level of BIM expression predicted the level of clinical response to EGFR inhibitors. The data therefore suggest that pretreatment BIM levels represent a functional biomarker that can be used to predict the apoptotic response of oncogene-addicted cancers to targeted therapy. ■

See article, p. 352.

Note: *In This Issue* is written by *Cancer Discovery* Science Writers. Readers are encouraged to consult the original articles for full details.

In This Issue

Cancer Discovery 2011;1:275.