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In silico and biological evaluation of correlation between efficacy of PI3K inhibitors with the activation status of signaling pathways in a panel of JFCR39 human cancer cell lines

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The phosphoinositide 3-kinase (PI3K) pathway is frequently activated in cancer and is considered a promising therapeutic target. Diverse agents targeting this pathway including PI3K/Akt/mTOR inhibitors have recently been developed, and some have already entered clinical trials. Gain-of-function mutations of *PIK3CA* and PTEN loss are believed to activate PI3K-downstream effectors. However, it is not well understood whether the efficacies of PI3K pathway inhibitors can be predicted based on these aberrations and on the activation status of other PI3K pathway members. To clarify this issue, we studied mutation, expression and phosphorylation of PI3K pathway members and those of RAS pathway members in a panel of pharmacologically well-characterized 39 human cancer cell lines (JFCR39). We also studied the in vitro efficacies of 25 PI3K pathway inhibitors in addition to conventional anticancer drugs, and then combined these data to construct an integrated database of pathway activation status and drug efficacies (JFCR39-DB). In silico analysis of JFCR39-DB enabled us to evaluate correlations between the statuses of pathway members and the efficacies of PI3K inhibitors, as well as those within pathway members and those within inhibitors. For example, phospho-Akt and *KRAS*/*BRAF* mutation prominently correlated with the efficacy and the inefficacy of PI3K inhibitors respectively, while *PIK3CA* mutation and PTEN loss did not. These correlations were confirmed in xenografted human tumors in vivo, suggesting that they could serve as predictive biomarkers for PI3K inhibitors. Therefore JFCR39-DB is a useful tool to identify predictive biomarkers, and to study molecular pharmacology of the PI3K pathway in cancer.

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Temozolomide treatment combined with Notch inhibition blocks recovery of glioma cells through the induction of senescence

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Gliomas are treated with a combination of surgery, radiation and temozolomide (TMZ), however these therapies do not result in a long-term cure. Our lab demonstrated that some glioma cells undergo a transient cell cycle arrest in response to chemotherapy. Treatment with TMZ decreases sphere formation; however, after a short recovery period, a small number of cells resume sphere formation and self-renewal, measured by secondary sphere formation. After TMZ treatment, we targeted the cells capable of recovery by suppressing Notch using gamma-secretase inhibitors (GSIs). TMZ+GSI treated cells do not recover and are no longer capable of self-renewal. TMZ+GSI synergy is dependent on the sequence of the drug treatments. Recovery was inhibited when GSI was administered 24 hrs after TMZ treatment. If the GSI was administered prior to, or concurrently with TMZ, the culture was able to recover and form secondary spheres. TMZ+GSI treatment also decreases tumorigenicity. When glioma cell lines were treated *in vitro* and implanted in immunodeficient mice, TMZ+GSI treatment resulted in extended latency and greatly increased survival. In addition, *in vivo* TMZ+GSI treatment completely blocked tumor progression in 50% of mice, while none of the TMZ-only treated mice survived. To determine the mechanism that TMZ+GSI blocks recovery, we analyzed cell death and senescence. TMZ+GSI treated cultures showed no increase in cell death, compared to TMZ-only cultures. Instead, we observed an increase in the number of cells expressing senescence-associated b-galactosidase. Gene expression was also analyzed after drug treatments to confirm the induction of senescence. p21 is upregulated in cells that have undergone either a transient cell cycle arrest or senescence. We found that both TMZ-only and TMZ+GSI treatments increased p21 expression beginning 2 days after treatment; however, in the TMZ-only cultures, p21 expression returned to basal levels 18 days after treatment, while p21 remained upregulated in the TMZ+DAPT cultures. This demonstrates that the addition of GSI treatment shifts TMZ-treated glioma cells from a transient cell cycle arrested state to a permanent senescent state. This data demonstrates the importance of the Notch pathway in chemoprotection and maintenance of TMZ-treated gliomas. The addition of GSIs to current treatments is promising target-directed therapy to decrease the rate of brain tumor recurrence by targeting cells to undergo senescence.

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Tivozanib activity in combination with capecitabine, 5-fluorouracil (5-FU), or docetaxel, in traditional or engineered subcutaneous breast tumor models

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Background: Taxanes and fluoropyrimidines are 2 major classes of chemotherapy drugs used to treat advanced breast cancer. Recent clinical data indicate that vascular endothelial growth factor (VEGF) signaling pathway inhibitors exhibit promising activity in combination with the 5-FU prodrug capecitabine. The antitumor activity of tivozanib, a potent and selective kinase inhibitor of VEGF receptors-1, -2, and -3, was evaluated alone and in combination with capecitabine in a HER2-engineered murine tumor that exhibits tivozanib resistance. In addition, tivozanib was evaluated alone and in combination with representative taxane and fluoropyrimidine chemotherapy drugs in a xenograft model of breast cancer.
Materials and Methods: Subcutaneous BH216 HER2-engineered murine or MX-1 human breast tumors were grown in female nude mice. Mice bearing HER2-engineered tumors were treated with oral tivozanib (5 mg/kg/day), capecitabine (300 mg/kg/day using a 14 days on, 7 days off schedule), or both drugs in combination. Mice bearing MX-1 tumors were treated with oral tivozanib (20 mg/kg/day), 5-FU (100 mg/kg weekly for 3 weeks) or docetaxel (10 mg/kg weekly for 3 weeks), or a combination of tivozanib and chemotherapy. Tumor growth inhibition (TGI) was calculated using standard criteria.
Results: In the HER2-engineered breast tumor model, treatment with tivozanib alone or capecitabine alone resulted in continued tumor growth with only modest TGI. However, the combination of tivozanib plus capecitabine led to complete growth inhibition. In the MX-1 xenograft model, tivozanib monotherapy exhibited robust TGI, and modest improvements in TGI were observed when tivozanib was combined with 5-FU or docetaxel relative to tivozanib monotherapy. Following discontinuation of tivozanib alone or in combination with 5-FU, tumor regrowth was observed, whereas mice treated with tivozanib plus docetaxel were tumor-free by Day 34, with no tumor recurrence at Day 63.
Conclusions: When examined in a tivozanib-resistant murine breast tumor model, tivozanib combined with capecitabine appeared to reverse tivozanib resistance. Tivozanib treatment also potently inhibited MX-1 breast tumor growth and enhanced the anticancer effects of 5-FU and docetaxel.

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Identification and characterization of specific inhibitors of USP7/HAUSP deubiquitinating enzyme

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Regulated protein turnover is primarily controlled by the ubiquitin-proteasome system. The only marketed drug related to the ubiquitin-proteasome system, bortezomib, is acting as a proteasome inhibitor and has been approved for the treatment of some hematological cancers. Targeting the upstream ubiquitin conjugation/deconjugation system carries out promises of therapeutics with increased specificity and selectivity. Ubiquitin-specific proteases (USP) are involved in the deubiquitination of specific target substrates regulating their stability, subcellular localization and/or activation status. USP represent a drugable target class due to their thiol-protease catalytic core which is amenable to pharmacological inhibition by small molecules. A genome-wide RNAi screen of the catalytically active human USPs in cancer-relevant cellular models and phenotypic assays allowed us to identify USP7/HAUSP as promising cancer target. Fluorescence-based screening assays using optimized USP substrates including various ubiquitin derivatives (ubiquitin precursor, branched ubiquitin chains) as well as specific, physiological substrates were developed. High-throughput screening performed on our chemically diverse library followed by different optimization programs resulted in the discovery of several series as novel USP7 inhibitors. Our progress made towards the specificity issue will be presented here with the identification of the first USP7-specific series. These will help further validate this novel class of molecular targets and may provide a structural basis for the development of new anticancer drugs.