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Enzastaurin (LY317615.HCl) suppresses signaling through the PKC and AKT pathways, inducing apoptosis, suppressing tumor-induced angiogenesis and reducing growth of human cancer xenografts

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PKC and PI3 Kinase/AKT pathway activation have been broadly implicated in human cancer development, motility, cell cycle progression, apoptosis, angiogenesis and chemoresistance. The bisindolylmaleimide, Enzastaurin (LY317615.HCl), was developed as an ATP-competitive inhibitor of PKCβ and was advanced to the clinic as an anti-angiogenic. In addition to its robust anti-angiogenic activity, we recently showed that Enzastaurin suppresses signaling through the PKC and AKT pathways, blocking phosphorylation of GSK3β, ribosomal protein S6, AKT and CREB and inducing tumor cell apoptosis. Accordingly, Enzastaurin induces apoptosis in a wide array of human cancer cells and suppresses growth of human cancer xenografts. As in cultured cells, GSK3βser9 phosphorylation is reduced in xenograft tissues and parallels a marked, time-dependent reduction in peripheral blood mononuclear cells harvested from these xenograft-bearing mice.
Enzastaurin has recently advanced to Phase III human clinical trials for the treatment of recurrent Glioblastoma Multiforme and Diffuse Large B Cell Lymphoma. To continue to support the progress of Enzastaurin in clinical trials, we have focused our pre-clinical studies on defining biomarkers for possible patient stratification, refining the mechanism of action and exploring the utility of Enzastaurin in combination with standard oncolytics and newer, targeted anti-cancer agents. Our data now show that Enzastaurin induces apoptosis through both intrinsic and extrinsic cell death pathways. Further, we show that pGSK3βser9 may provide a rational, mechanism-based biomarker for Enzastaurin activity and, possibly, patient selection. Finally, our preliminary data have revealed that Enzastaurin may be effective in combination with standard oncolytics as well as targeted therapies.

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Discovery of a novel anti-tumor agent targeting NF-kB pathway

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NF-κB is a major transcription factor for angiogenesis-related genes (MMP-9 and IL-8) and anti-apoptotic genes. Recent studies suggest that inhibition of NF-κB function could be an attractive target for anticancer drugs, and then some NF-κB inhibitors are currently in development as a new molecular therapeutics. We have synthesized a group of indazole derivatives and evaluated their potentials as anticancer agents. Here we report the discovery and characterization of a novel anti-tumor agent DYA-686, a small molecular weight inhibitor targeting NF-κB pathway.
DYA-686 was found through a screening where inhibition of PMA-stimulated MMP-9 production in immortalized human endothelial cells was assayed by using gelatin zymography. The compound dose-dependently inhibited TNF-α-induced IL-8 production in EBC-1 (a human NSCLC cell line) and pancreatic HPAC cells and suppressed NF-κB-dependent transcriptional activity in HPAC cells by luciferase-reporter assay. The compound also showed direct inhibitory effects on proliferation of various cancer cell lines including EBC-1, HPAC and rat glioma C6 cells. Furthermore, oral daily dosing of DYA-686 to nude mice resulted in inhibition of C6-induced intradermal neovascularization as determined by counting the number of blood vessels by light microscopy and also potently inhibited in vivo sc growth of EBC-1 and C6 tumor xenografts. Whereas DYA-686 showed no inhibitory effects on nuclear translocation of p65 of NF-κB and on the activities of 156 kinases including IKKs in the KinaseProfiler screening, the compound suppressed the expression of p65 in EBC-1 cells on immunoblot analysis.
These results suggest that DYA-686 is an inhibitor of NF-κB pathway with a unique mechanism of action, exerting anti-angiogenic and anti-tumor activities.

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Analysis of small molecule Ras/Raf interaction inhibitors in C. elegans identifies both on-target and off-target activities

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A key goal of anticancer drug discovery is to rapidly uncover and identify both on-target and off-target activities of potential new drugs. To evaluate compounds targeting oncogenic forms of the small GTPase Ras and a major downstream effector, the serine/threonine kinase Raf, we have used the nematode C. elegans to report on the activity and selectivity of putative Ras/Raf interaction inhibitors in vivo. The conserved Ras/Raf/MAPK signaling pathway regulates vulval formation; a gain-of-function mutation in the Ras homolog LET-60 (let-60 gf), similar to the G12V mutation in oncogenic Ras, causes a quantifiable multivulva (Muv) phenotype. Gf mutations in LIN-45 (Raf homolog) can substitute for LET-60. The Muv phenotype can thus be used as a readout for excessive activity of Ras/Raf signaling, and genetic epistasis experiments can determine the level of inhibition for pharmacologically active agents.
Small molecule Ras/Raf interaction inhibitors were identified in a yeast two-hybrid screen, confirmed in vitro, and shown to reverse Ras transformation in cell-based assays. We tested if the inhibitors disrupted the Muv phenotype in let-60-gf worms (activated Ras) but not in lin-45-gf worms (activated Raf). Three different Ras/Raf inhibitors, MCP-110, MCP-116 or MCP-146, caused significant and dose-dependent reductions in the Muv phenotype of let-60-gf worms, whereas the negative control (MCP122) did not. In addition, lin-31(n301) (tissue-specific MAPK pathway transcription factor) worms were resistant.
Interestingly, two MCP compounds also caused a switch from solitary to social feeding behavior that is also seen in loss-of-function mutations in NPR-1, a G-protein coupled receptor (GPCR) that is the worm homolog of neuropeptide Y receptor. These two MCP compounds may thus have additional off-target activity that results in blocking NPR-1, a supposition that is currently being tested. Our results support the notion that off-target activities that would not be uncovered in cell-based assays can be identified using simple in vivo preclinical models. Desirable off-target activity might be developed separately for future use. These studies thus contribute to identifying which MCP compounds to pursue for future development, and further support the use of C. elegans as a tool to demonstrate the potency and selectivity of drugs designed to specifically target the Ras/Raf interaction or other molecularly targeted anticancer candidates.

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mTORC1 inhibition with rapamycin leads to activation of PI3K/AKT signalling via an mTORC2 dependent mechanism in melanoma cells

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Background: The mTOR inhibitor rapamycin has been clinically studied as a novel approach for the treatment of advanced malignant melanoma with only limited success. In cancer cells, inhibition of mTORC1 may lead to activation of AKT. Therefore, we studied the signalling pathways modulated by rapamycin in malignant melanoma and evaluated compounds for combination therapy with rapamycin.
Methods: Mel-Juso and 518A2 melanoma cell lines were assessed for cell viability, cell death, and modulation of the cell cycle. Expression and phosphorylation status of mTOR pathway components were quantified using Western blotting. For combination therapy, rapamycin was combined with the PI3K inhibitor LY294002 and a siRNA directed against rictor.
Results: Rapamycin demonstrated limited anti-tumor activity in the two melanoma cell lines. On a molecular level, rapamycin inhibited phosphorylation of the well-established mTOR targets S6K1 and 4E-BP1, but also led to massive phosphorylation of AKT suggesting the activation of a feedback loop. Interestingly, LY294002 alone also led to enhanced AKT phosphorylation after prolonged treatment. Inhibition of rictor via siRNA transfection led to reduced p-AKT levels in cells that have been stimulated with rapamycin or LY294002 pointing towards a role of mTORC2 in the feedback activation of AKT. Combination of rapamycin and LY294002 resulted in synergistic reduction of cell viability, G1/G0 cell cycle arrest and downstream target phosphorylation. Surprisingly, combination of LY294002 at high concentrations with rapamycin interrupted the feedback activation, whereas combination of LY294002 at lower concentrations did not reduce AKT phosphorylation. PTEN status of the examined cell lines did not influence the AKT feedback loop significantly.
Conclusion: Our data suggest that inhibition of mTORC1 leads to activation of PI3K/AKT in melanoma cells. The mTORC2 protein is