Results: We determined that TLN-4601 potently inhibited the anchorage-dependent and -independent growth of KRAS-transformed human pancreatic nestin-positive (HPNE) duct-derived cells. We also found that the growth of KRAS mutation-positive pancreatic carcinoma cell lines was inhibited by TLN-4601. We then assessed the ability of TLN-4601 to antagonize RAS signal transduction. Consistent with the ability to directly antagonize RAS, we found that TLN-4601 treatment caused cell context-dependent reduction in RAS and RAF-1 protein expression and an inhibition of p70 S6 kinase and MEK1/2 phosphorylation.
Conclusions: Our results support the use of TLN-4601 for pancreatic cancer treatment and are consistent with a model where the anti-tumor activity of TLN-4601 is mediated, in part, through antagonism of RAS signaling. The mechanism of action of TLN-4601 is cell context-dependent and is associated with antagonism of multiple facets of Ras signal transduction.

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RhoA and RhoB inversly modulate estrogen receptor alpha expression and transcriptional activities in breast cancer cell lines

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Background: About two thirds of mammary tumors express estrogen receptor alpha (ERα) and hormonotherapy is then recommended. Nevertheless, there are systematically resistances to these treatments that impose the search for new pharmacological targets. Estrogens act mainly through the well-known ERα. However, cross-talks have been clearly demonstrated between ER and growth factors signalling pathways. Ras family proteins, such as Rho proteins, are key elements in those cross-talks. RhoA is frequently overexpressed in breast cancers and has been shown to down-regulate ER-mediated transcription. Our purpose was then to decipher the effect of Rho proteins inhibition on ERα expression and transcriptional activities.
Material and Methods: We specifically abolished the expression of either RhoA or RhoB proteins using two independent sequences of interfering RNA for each protein in MCF-7, MELN, T47D, ZR75 cells (hormonodependent breast cancer cell lines) and in LCC2 and LCC9 cells (hormonoresistant breast cancer cell lines). We then studied the impact of RhoA and RhoB inhibition on the one hand on ER target gene expression (by RTq-PCR or by a luciferase assay) and on the other hand, on ERα expression in cell model. Finally, we analyzed ER expression in RhoB knock out mice.
Results: We first showed in MCF-7 cells that RhoA inhibition increases both the expression of a luciferase reporter gene controlled by the vitellogenine Estrogen Responsive Element and the Progesteron Receptor (PR) mRNA. The inhibition of RhoA also increases ERα expression both at the mRNA and proteins levels. On the contrary, the inhibition of RhoB decreases the expression of the luciferase reporter gene controlled by the vitellogenine ERE and PR mRNA in MCF-7. Besides, RhoB inhibition decreases ERα expression in MCF-7, TR47D, ZR75, LCC2 and LCC9 cells. We also confirmed this result in Mouse Embryonnic Fibroblasts (MEFs) from RhoB knock-out mouse.
Conclusion: In brief, our results evidence RhoA and RhoB participation in the balance of expression of ER and in the individual modulation of the expression of various target genes. Further investigations, especially experiments in hormonoresistant cells, are now necessary for a better understanding of hormonoresistance.

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Inhibition of protein kinase C as the molecular basis of the synergism between safingol and irinotecan in colon cancer treatment

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Background: Safingol is a synthetic sphinganine which has been developed as a protein kinase C (PKC) inhibitor, and is currently evaluated in Phase I clinical trials. As PKC has been found in elevated levels in colon cancer cells, the aim of this study was to investigate the effects of safingol on colon cancer cell viability and its potential to enhance the cytotoxic effect of irinotecan for colon cancer therapy.
Materials and Methods: The anti-cancer effects of safingol as single agent or in combination with irinotecan in HT-29 and LS-174T colon cancer cells were determined using MTT assay. The combination index (C.I.), based on the median effect principle by Chou and Talalay, was computed to determine drug synergism. Treated cells were stained with annexin-V/7AAD to determine the extent of apoptosis. The expression levels of phosphorylated PKC and its downstream substrate, MARCKS, were determined using Western blot.
Results: As a single agent, safingol reduced colon cancer cell viability in a concentration-dependent manner, with IC_{50} values of $2.5\pm1.1\,\mu M$ and $3.4\pm1.0\,\mu M$ in HT-29 and LS-174T, respectively. Over 50% of treated HT-29 cells underwent apoptosis after a 48-h exposure to $10\,\mu M$ safingol. Interestingly, 24.9% of treated cells were annexin-V^- but $7AAD^+$, suggesting the possibility of necrosis or other death mechanisms. Further studies with the pan-caspase inhibitor, Z-VAD-FMK, indicated that cell death was not prevented in safingol-treated cells, indicating that safingol exerted its cytotoxicity via a caspase-independent mechanism. A 1:1 (mol/mol) combination of safingol/irinotecan was synergistic in both HT-29 and LS-174T, with C.I. values <1.0. This combination enabled significant dose reduction of irinotecan, with 4-fold and 250-fold reduction in HT-29 and LS-174T, respectively. Although safingol was developed as a PKC inhibitor, no decrease was observed in the expression of p-PKC or the downstream substrate p-MARCKS with $10\,\mu M$ safingol. However, treatment with safingol/irinotecan combination was associated with decreased expression of p-PKC and p-MARCKS, suggesting a possible molecular basis for the observed synergistic effect.
Conclusions: Our results show that inhibition of PKC by safingol/irinotecan combination could be a potentially effective strategy for colon cancer treatment. Future *in vivo* studies are warranted to further explore the therapeutic potential of this drug combination.

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An acquired point mutation in MEK2 causes resistance to allosteric MEK inhibitors

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Mutations in the ATP binding site are emerging as a common acquired resistance mechanism for ATP competitive kinase inhibitors, however potential resistance mechanisms for allosteric kinase inhibitors are poorly understood. We studied drug resistance mechanisms for an allosteric MEK inhibitor by generating a drug-resistant cell line in vitro. Exposure of the k-ras mutant colon cell line HCT116 (gIC50=2 nM) to increasing concentrations of the MEK inhibitor GSK1120212 led to isolation of a drug resistant population capable of growing under high concentration ($1\,\mu M$) of drug (gIC50 > $7\,\mu M$). The drug resistant population was also resistant to other allosteric MEK inhibitors while remaining sensitive to inhibitors of other targets (KSP and PI3K). Clones were isolated and several MAPK pathway related genes were sequenced. A single point mutation in MEK2 resulting in the amino acid change L119P was identified. MEK2-L119 is located within the allosteric binding site for GSK1120212 as well as other reported MEK inhibitors (PD0325901 and AZD6244). siRNA to MEK2 reduced levels of MEK2-L119P but not MEK1 and re-sensitized these cells to GSK1120212. The homologous MEK1 L115P mutant construct was engineered to test whether it could similarly confer resistance to GSK1120212. Exogenous expression of the MEK1-L115P mutant but not MEK1-wt was demonstrated to confer drug-resistance to tumor cell lines sensitive to GSK1120212. Finally, HCT116 (MEK2-L119P) formed tumors in mice that were relatively resistant to GSK1120212 compared to wild type HCT116 tumors. These data demonstrate that resistance to MEK inhibitors including GSK1120212 can be caused by a mutation of MEK2-L119P or MEK1-L115P permitting phosphorylation of ERK in presence of drug. To date, these mutations or polymorphisms have not been identified in clinical tumor samples.

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Analysis of MAP kinase signalling pathway in KIT & PDGFRA wild-type GISTs

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Background: Gastrointestinal stromal tumours (GISTs) are commonly driven by oncogenic mutations in KIT and PDGFRA genes, which are important molecular targets to specific kinase inhibitors, such as imatinib mesylate. However, 10−40% of GISTs patients are wild-type for KIT and PDGFRA genes. The prognostic significance of wild-type GISTs is controversial, and they rarely respond to imatinib mesylate. MAPK pathway is implicated in some tumor types through alterations in RAS, RAF or RKIP (Raf Kinase Inhibitor protein) molecules. Few studies have investigated the