of these genes reveals a cohesive perturbation of the cholesterol and fatty acid biosynthesis pathway. Genes from this pathway (~21) were upregulated (>1.8-fold) and include 3-hydroxy-3-methylglutaryl-Coenzyme A (HMGCoA) synthase 1 and reductase, low density lipoprotein receptor (LDLR), and squalene epoxidase among others. As this was consistent with published gene expression changes for bafilomycin, we measured the transcriptional consequences of bafilomycin treatment on these cells, and found correlations between the ~900 genes dysregulated by the two drugs of PCC > 0.9 for UACC62 and PCC > 0.75 for LOX. Bafilomycin has been reported to inhibit cholesteryl ester synthesis through sequestration of free cholesterol in the endosomal/lysosomal compartment as a consequence of V-ATPase inhibition. The high degree of coherence between their gene profiles supports the premise that palmerolide is a V-ATPase inhibitor, and consequently has an effect on cholesterol sequestration.
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Natural products

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JNK-mediated p53 phosphorylation and stabilization contributes to the sensitization effect of luteolin on the anti-cancer effect of cisplatin

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Background: Luteolin is a flavonoid widely present in edible plants. Our previous studies have demonstrated the sensitization activity of luteolin on cancer cell apoptosis induced by TNFα or TRAIL. In this study we further investigate the synergistic effect of luteolin on cisplatin-induced apoptosis and the molecular mechanisms involved.
Material and Methods: Human cancer cells were pretreated with luteolin, followed by cisplatin. Apoptotic cell death, p53 protein level and JNK activation were determined using various methods.
Results: First, we provided evidence that apoptosis-induced by combined treatment of luteolin and cisplatin is p53-dependent: only p53 wild type cancer cells, such as HCT116 and HepG2, but not the p53 mutant cancer cells, such as HT29 and Hep3B, were sensitive to luteolin and cisplatin. Further, knock down of p53 protein level by siRNA made p53 wild type cancer cells resistant to luteolin and cisplatin, indicating a critical role of p53 in the sensitization process. Second, we observed significant increase of p53 protein level in luteolin-treated cancer cells, without increase of p53 mRNA level, indicating the possible effect of luteolin on p53 post-transcriptional regulation. Third, we found the critical role of c-Jun-N-terminal kinase (JNK) in luteolin-mediated p53 protein stablilization: luteolin activates JNK and JNK then stabilizes p53 via phosphorylation, leading to reduced ubiquitination and proteasomal degradation. Finally, by using an *in vivo* nude mice model xenografted with HCT116 cells, we confirmed that luteolin enhanced the cancer therapeutic activity of cisplatin via p53 stabilization and accumulation.
Conclusions: Data from this study demonstrate that luteolin enhances the anti-cancer activity of cisplatin via JNK-mediated p53 phosphorylation and stabilization. Our study thus supports the potential clinical application of luteolin as a chemosensitizer in cancer therapy.

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Preclinical development of novel betulinic acid derivatives as potent anticancer and antiangiogenic agents for systemic administration

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Introduction: Betulinic acid (BA) is a natural pentacyclic lupane-type triterpene shown previously to have potent anti-cancer activity in melanoma and neuroectodermal cancers. We have demonstrated the potential of novel C-2, C-3, C-20 and C-28 modified BA derivatives as broad-spectrum anti-cancer and anti-angiogenic agents.
Material and Methods: Cytotoxicity of novel derivatives was studied on a panel of human tumor cell lines using the tetrazolium-based MTT assay and *in vivo* efficacy was evaluated in melanoma and ovarian xenograft models. The *in vitro* anti-angiogenic effect was studied using human endothelial cells while anti-metastatic effect was tested in the mouse lung nodule assay. Predictive absorption, distribution, metabolism, elimination and toxicity (ADMET) studies were done using commercially available and validated software. Lead development studies like solubility, permeability, metabolic stability, cytochrome P450 inhibition and plasma protein binding were done using standard methods. Single dose pharmacokinetic study was carried out in rats and results analyzed using WinNonlin v5.0.1. Safety studies were done in rodents upon intravenous administration of potent derivatives.
Results: More than 1500 derivatives were screened and about 30 derivatives showed better broad-spectrum anti-cancer activity compared to BA in melanoma, glioblastoma, lung, and ovarian cancers with better selectivity for cancer cells and endothelial cells compared to normal cells. Modifications at C-3 position resulted in more potent derivatives. These derivatives, at non-cytotoxic concentration, significantly ($P < 0.05$) inhibited chemotaxis of endothelial cells towards angiogenic factors. Efficacy studies demonstrate that potent derivatives inhibit growth of human tumor xenografts and the formation of melanoma lung nodules in athymic mice. ADMET studies show that BA derivatives have poor solubility (<0.1 μg/ml), low to moderate permeability ($\log P_e < -5.0$) and high protein binding (>90%) suggestive of low/moderate bioavailability. A few derivatives had good *in vitro* metabolic stability (>90%). None of the derivatives inhibited key cytochrome P450 enzyme isoforms *in vitro* (IC_{50} > 10 μM) indicating less potential for drug interaction in combination therapy. The derivatives were safe in animals at the therapeutic dose and possess favorable properties of a systemically administered drug in the pharmacokinetic study.
Conclusion: Appropriate modifications in BA have resulted in more potent anti-cancer and anti-angiogenic compounds. ADMET studies indicate that BA derivatives have potential for development in a suitable anti-cancer formulation. Being natural-product derived compounds with good activity and low toxicity BA derivatives are potential anti-cancer agents.

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Curcumin inhibits tumor growth and angiogenesis in glioblastoma xenografts

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Background: Among the natural products that have shown chemopreventive and anticancer properties, curcumin is one of the most potent. In the current study, we investigated the effects of this natural product on growth of human gliomas U-87 cells xenograft in immunodeficient *nu/nu* mice.
Material and Methods: The anti-proliferative effect of curcumin on human glioma cell line U87 was studied in vitro by ^{3}H-thymidine incorporation methods. Tumor size and animal survival time were followed in curcumin treated mice with subcutaneous (s.c.) gliomas. Furthermore, *in vitro* proliferation, migration and tube formation were assayed on rat brain capillary endothelial cells to explore the effect of curcumin on angiogenesis.
Results: Curcumin was demonstrated to exert anti-proliferatif effects of human gliomas cells in a dose-dependent manner (IC50 = 12 μM). In addition, curcumin (50 mg/kg/day) exert significant antitumor effects on s.c. gliomas including slower tumor growth rate (up to 70%) and higher animal survival rate (up to 40%). Furthermore, treatment with curcumin inhibits angiogenesis, as indicated by the concentration of hemoglobin in the tumor. *In vivo* experiments revealed that curcumin decrease matrix metalloproteinase-2 (MMP-2) activation, whereas MMP-9 activation is unaffected by this natural product. Our study also shows that curcumin inhibited proliferation of endothelial cells *in vitro* (IC50 = 9 μM). In tube formation and cell migration assays using brain capillary endothelial cells, noncytotoxic doses of curcumin significantly inhibited formation of intact tube networks and reduced the number or migratory cells.
Conclusions: Our results indicate that, curcumin caused significant antitumor effects and inhibited angiogenesis in s.c. gliomas. Thus, curcumin might be helpful for the prevention and treatment of gliomas.

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Safety profile of ECO-4601, a novel PBR ligand anticancer agent, in primates

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Background: ECO-4601 is a structurally novel farnesylated dibenzodiazepinone (MW 462) discovered through Ecopia's Decipher® technology proprietary drug discovery platform. Initial *in vitro* assessment indicated cytotoxic activity against a wide panel of tumor cell lines, including several brain tumor cell lines. The mechanism of action of ECO-4601 is unknown at this time. However, the product binds selectively to the peripheral benzodiazepine receptor (PBR), preferentially expressed in tumors, with