A1
Lead Compound Discovery and Rapid Pharmacokinetic Modeling of Botanical Medicines for Hormone Replacement Therapy
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Aim: Like other traditional herbal medicines, the bioactive compounds in many botanical extracts consumed for menopausal health is not known. Furthermore, unlike single-entity drugs whose presence in the body fluids can be measured by spectrometric methods, an appropriate technological platform for in vivo tracking of dozens of steroid active compounds following administration of botanical extracts is lacking.

Methods: Using the progesterone receptor as a target, we developed a sensitive progesterone-responsive bioassay, screened traditional Chinese medicinal herbal extracts, and discovered Ligusticum chuanxiong (LC) with significant progestogenic activity.

Results: Bioassay-guided fractionation resulted in the isolation of 2 novel phyto-progestogens, the most potent being dihydrodiligustilide (0.02% of crude extract, EC50 of ~90 nM) a dimer of a major LC constituent, Z-ligustilide. Using the same progesterone-responsive bioassay, we determined that an ethanolic LC extract rendered a sensitive progesterone-responsive bioassay, screened traditional Chinese medicinal herbal extracts, and discovered Ligusticum chuanxiong (LC) with significant progestogenic activity.

Conclusion: Our data indicate that relatively simple receptor-driven bioassays are useful for the discovery of novel bioactive compounds, and detecting summated steroidogenic activities of compounds both known and unknown, in pharmacokinetic/pharmacodynamic analysis of complex herbal mixtures.

A2
The Role of 5'-Untranslated Region in Translational Suppression of Tumour Suppressor Gene OKL38 in Hepatocellular Carcinoma
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Aim: Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. OKL38 is a recently discovered pregnancy-induced growth inhibitory gene whose expression is significantly reduced in various breast cancer cell lines and kidney tumour. The objective of this study was to determine the expression and decipher the mechanism of the loss of OKL38 in HCC.

Methods: Immunohistochemistry was performed on 92-paired HCC and adjacent benign liver tissues. Western blot analysis and one-step RT-PCR were performed on 28 and 19 pairs of these tissues, respectively. Transfection of Chang liver cells with various OKL38 constructs was performed to determine the translational regulation of its 5'-untranslated region.

Results: OKL38 protein was lost or reduced in 64.2% (18 of 28) of the HCC as compared to adjacent benign tissues and in all liver cancer cell lines examined. Immunohistochemical analysis demonstrated that OKL38 protein was undetected in 41.3% (38 of 92) of HCC, while 39.1% (36 of 92) of HCC showed weak staining. The lost or reduced expression of OKL38 correlated with high tumour stages (P = 0.0042) as determined by non-parametric trend analysis. Over-expression of the OKL38 and OKL38-eGFP fusion protein in Chang liver cells led to cell death. Importantly, we discovered that the 5'-untranslated region of OKL38 played a critical role in regulating translation of its mRNA.

Conclusion: Our data suggest that the loss of this protein may lead to the development and/or progression of HCC. A better understanding of the function of OKL38 in normal liver and during hepatocarcinogenesis may lead to the development of new preventative and therapeutic modalities for HCC.

A3
Luteolin Sensitises Tumour Necrosis Factor-related Apoptosis-inducing Ligand (TRAIL)-induced Apoptosis in Cancer Cells by Inhibiting Protein Kinase C and Down-regulating X-linked Inhibitor of Apoptosis Protein (XIAP)
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Background: Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is an important member of the TNF superfamily, with great potential in cancer therapy. However, its potential application is limited as many cancer cells are found to be resistant to the cytotoxicity of TRAIL. Luteolin is a flavonoid rich in diets and some medicinal plants.

Aim: To evaluate whether luteolin sensitises TRAIL and elucidate the mechanism involved.

Results: Pretreatment with a non-cytotoxic concentration of luteolin significantly sensitised TRAIL-induced apoptosis in TRAIL-resistant cancer cells. Such sensitisation is achieved through enhanced caspase-8 activation and caspase-3 maturation. Further, the protein level of X-linked inhibitor of apoptosis protein (XIAP) was markedly reduced in cells treated with luteolin and TRAIL, and ectopic expression of XIAP protected the cell death induced by luteolin and TRAIL, showing that luteolin sensitises TRAIL-induced apoptosis through down-regulating XIAP. In search of the molecular mechanism responsible for XIAP down-regulation, we found that luteolin and TRAIL promoted XIAP ubiquitination and proteasomal degradation. Next, we demonstrated that protein kinase C (PKC) activation prevented cell death induced by luteolin and TRAIL via suppression of XIAP down-regulation. Moreover, luteolin inhibited PKC activity and bisindolylmaleimide I (BIM), a general PKC inhibitor, simulated luteolin in sensitising TRAIL-induced apoptosis.

Conclusion: These results present a novel anticancer effect of luteolin and support its potential application in cancer therapy in combination with TRAIL. In addition, our data reveal a new function of PKC in cell death: PKC activation stabilises XIAP and thus suppresses TRAIL-induced apoptosis.
Reduced Susceptibility to DEN-induced Hepatocarcinogenesis in C/EBPα Knock-in Mice

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Aim: The purpose of this study was to investigate whether the expression of transgenic CCAAT/enhancer binding protein alpha (C/EBPα) confers a degree of protection against hepatocellular growth.

Methods: Our lab has successfully created a C/EBPα knock-in (KI) mouse strain by placing a single-copy C/EBPα transgene under the control of 1 of the 2 alleles of the endogenous mouse α-fetoprotein (AFP) gene promoter. Diethylnitrosamine (DEN) was used to induce HCC in both wild type (WT) and KI animals.

Results: KI mice developed half the number of hepatocellular nodules as compared to WT mice. Immunohistochemistry showed reduced C/EBPα content in WT nodules whereas KI nodules stained strongly for C/EBPα. Nuclear p21 was absent in WT nodules whereas cytoplasmic p21 was abundant; KI nodules were positive for nuclear p21. Interestingly, only C/EBPα positive nodules were positive for nuclear p21, suggesting that C/EBPα may be required for p21 nuclear localisation to inhibit growth.

Conclusion: Our data establish that the reactivation of C/EBPα expression during hepatic carcinogenesis can inhibit liver tumour growth in vivo.