The University of Hong Kong

1. **Shotgun Proteomics and Quantitative Pathway Analysis of the Mechanisms of Action of Dehydroeffusol, a Bioactive Phytochemical with Anticancer Activity from *Juncus effuses*.**

   S. Chang, L. K. Sy, B. Cao, C. T. Lum, W. L. Kwong, Y. M. Fung, C. N. Lok, C. M. Che.

   Journal of Proteome Research., 2018, 17, 2470–2479.

   Abstract

   Dehydroeffusol (DHE) is a phenanthrene isolated from the Chinese medicinal plant Juncus effusus. Biological evaluation of DHE reveals in vitro and in vivo anticancer effects. We performed a shotgun proteomic analysis using liquid chromatography-tandem mass spectrometry to investigate the changes in the protein profiles in cancer cells upon DHE treatment. DHE affected cancer-associated signaling pathways, including NF-κB, β-catenin, and endoplasmic reticulum stress. Through quantitative pathway and key node analysis of the proteomics data, activating transcription factor 2 (ATF-2) and c-Jun kinase (JNK) were found to be the key components in DHE's modulated biological pathways. Based on the pathway analysis as well as chemical similarity to estradiol, DHE is proposed to be a phytoestrogen. The proteomic, bioinformatic, and chemoinformatic analyses were further verified with individual cell-based experiments. Our study demonstrates a workflow for identifying the mechanisms of action of DHE through shotgun proteomic analysis.

2. C.-M. Che, L.-K. Sy, C.-N. Lok and W.-P. Lee. **Osteogenic compounds**. US Prov Appln No. 62/464,033, filed on 27 February, 2017

   The invention concerns the use of purified compounds (F1 and F4) from a natural Chinese medicinal herb in stimulating bone formation for treatment of osteoporosis.

3. **IRE1α Inhibition by Natural Compound Genipin on Tumour Associated Macrophages Reduces Growth of Hepatocellular Carcinoma.**

   H.Y. Tan, N. Wang, S.W. Tsao, C.M. Che, M.F. Yuen, Y.B. Feng.

   Oncotarget, 2016, 7, 43792-43804.

   Abstract

   Accumulating evidences postulated the influential roles of macrophages in mediating hepatocellular carcinoma (HCC) initiation and progression. In this study, we demonstrate that a small molecule, genipin reduced HCC growth through suppressing IRE1a-mediated infiltration and priming of tumour associated macrophages (TAMs). Oral administration of genipin (30mg/kg/2days) suppressed orthotopic HCC tumour growth without challenging the viability and proliferation of HCC cells. Genipin reduced infiltration of inflammatory monocytes into liver and tumour thereby suppressed TAMs presence in HCC microenvironment. Suppression of HCC growth was diminished in HCC-implanted mice with depletion of TAMs by liposome clodronate. Genipin inhibited the TAMs

migration, and reduced expression of TAMs-derived inflammatory cytokines that favors HCC proliferation. This is revealed by the in vivo deletion of IRE1a on TAMs in genipin-treated HCC-implanted mice. Diminishing IRE1a neutralised the inhibitory effect of genipin on TAMs. Silencing the expression of IRE1a greatly reduced TAMs migration and expression of inflammatory cytokines that prime HCC proliferation. Suppression of IRE1a led to reduced XBP-1 splicing and NF-κB activation. The reduced association of IRE1a with TRAF2 and IKK complex may be responsible for the genipinmediated inactivation of NF-κB. The findings show the important role of TAMs in inhibitory effect of genipin on HCC, and TAMs-expressing IRE1a as a promising target for disrupting the tumour environment that favor of HCC development.

4. Identification of "sarsasapogenin-aglyconed" timosaponins as novel Aβ lowering modulators of amyloid precursor protein processing

L.K.Sy, C.N. Lok, J.Y. Wang, Y. Liu, L. Cheng, P.K. Wan, C.T. Leung, B. Cao, W.L. Kwong, R.C.C. Chang, C.M. Che

Chemical Science, 2016, Advance article. ***DOI:*** *10.1039/C5SC02377G, Edge Article*

Abstract

The inhibition of amyloid β peptide (Aβ) production is a key approach in the development of therapeutics for the treatment of Alzheimer's disease (AD). We have identified that timosaponins consisting of sarsasapogenin (SSG) as the aglycone can effectively lower the production of Aβ peptides and stimulate neurite outgrowth in neuronal cell cultures. Structure-activity relationship studies revealed that the cis-fused AB ring, 3β-configuration, spiroketal F-ring and 25S-configuration of SSG are the essential structural features responsible for the Aβ lowering effects and neurite-stimulatory activities. New synthetic derivatives which retain the SSG scaffold also exhibited an Aβ lowering effect. Treatment of cells with timosaponins led to modulation of amyloid precursor protein (APP) processing through suppression of β-cleavage and preferential lowering of the production of the 42-amino acid Aβ species (Aβ42) without affecting another γ-secretase substrate. The SSG and "SSG-aglyconed" timosaponins also penetrated brain tissue and lowered brain Aβ42 levels in mice. Our studies demonstrate that timosaponins represent a unique class of steroidal saponins which may be useful for the development of AD therapeutics.

5. Autophagy-induced RelB/p52 activation mediates tumour-associated macrophage repolarisation and suppression of hepatocellular carcinoma by natural compound baicalin

H.Y. Tan, N. Wang, K. Man, S.W. Tsao, C.M. Che, Y. Feng

Cell Death and Disease, 2015, 6, e1942.

Abstract

The plasticity of tumour-associated macrophages (TAMs) has implicated an influential role in hepatocellular carcinoma (HCC). Repolarisation of TAM towards M1 phenotype characterises an immune-competent microenvironment that favours tumour regression. To investigate the role and mechanism of TAM repolarisation in suppression of HCC by a natural compound baicalin, Orthotopic HCC implantation model was used to investigate the effect of baicalin on HCC; liposome-clodronate was introduced to suppress macrophage populations in mice; bone marrow-derived monocytes (BMDMs) were induced to unpolarised, M1-like, M2-like macrophages and TAM using different conditioned medium. We observed that oral administration of baicalin (50 mg/kg) completely blocked orthotopic growth of implanted HCC. Suppression of HCC by baicalin was diminished when mice macrophage was removed by clodronate treatment. Baicalin induced repolarisation of TAM to M1-like phenotype without specific toxicity to either phenotype of macrophages. Baicalin initiated TAM reprogramming to M1-like macrophage, and promoted pro-inflammatory cytokines production. Co-culturing of HCC cells with baicalin-treated TAMs resulted in reduced proliferation and motility in HCC. Baicalin had minimal effect on derivation of macrophage polarisation factors by HCC cells, while directly induced repolarisation of AM and M2-like macrophage. This effect was associated with elevated autophagy, and transcriptional activation of RelB/p52 pathway. Suppression of autophagy or RelB abolished skewing of baicalin-treated TAM. Autophagic degradation of TRAF2 in baicalin-treated TAM might be responsible for RelB/p52 activation. Our findings unveil the essential role of TAM repolarisation in suppressive effect of baicalin on HCC, which requires autophagy-associated activation of RelB/p52.

6. Recent advances in ginseng as cancer therapeutics: a functional and mechanistic overview.

A.S. Wong, C.M. Che, K.W. Leung.

Natural Product Reports, 2015, 32, 256-72

Abstract

Covering: 2000 to 2014 Cancer is one of the leading causes of death worldwide. Ginseng, a key ingredient in traditional Chinese medicine, shows great promise as a new treatment option. As listed by the U.S. National Institutes of Health as a complementary and alternative medicine, its anti-cancer functions are being increasingly recognized. This review covers the mechanisms of action of ginsenosides and their metabolites, which can modulate signaling pathways associated

with inflammation, oxidative stress, angiogenesis, metastasis, and stem/progenitor-like properties of cancer cells. The emerging use of structurally modified ginsenosides and recent clinical studies on the use of ginseng either alone or in combination with other herbs or Western medicines which are exploited as novel therapeutic strategies will also be explored.

7. Novel mechanism of XIAP degradation induced by timosaponin AIII in hepatocellular carcinoma

N. Wang, Y.B. Feng, M.F. Zhu, K.M. Ng, C.M. Che

Biochimica et Biophysica Acta, 2013, 1833, 2890–2899

Abstract

Inducing tumor cell death is one of the major therapeutic strategies in treating cancer. The aimof this study is to investigate the mechanism underlying the involvement of autophagy in cell death induced by timosaponin AIII (TAIII). Cell viability was determined by MTT and cologenic assay; apoptosis was determined by flow cytometry and TUNEL assay; autophagy was examined by immunoblotting and immunofluorescence; ubiquitination was detected by co-immunoprecipitation; mRNA expression was detected by real-time PCR; and determination of necrotic cell death was approached with LDH assay. The in vivo tumor growth inhibition was determined by xenograft model. TAIII exhibits potent cytotoxicity on human hepatocellular carcinoma (HCC) cells without severe hepatic toxicity. TAIII induced caspase-dependent apoptosis in HCC, and the induction of apoptosis was attributed to the inhibition of TAIII on XIAP expression. Repressing XIAP expression allowed cell tolerance toward the treatment with TAIII. The suppression of XIAP by TAIII is under post-transcriptional control and independent of proteasomal-driven proteolysis. Instead, TAIII-induced AMPKα/mTOR-dependent autophagy was responsible for XIAP suppression and triggered the XIAP heading lysosomal degradation pathway. Ubiquitination of IAPs is required for the autophagic degradation induced by TAIII. Blockade of autophagy turns on the switch of necrotic cell death in TAIII-treated cells. Timosaponin AIII induces HCC cell apoptosis through a p53-independent mechanisminvolving XIAP degradation through autophagy–lysosomal pathway. The possibility of developing TAIII as a new anti-tumor agent is worth considering.

8. L. K. Sy, C. N. Lok and C. M. Che. “Timosaponin compounds”. PCT/CN2013/073666

9. Activation of autophagy of aggregation-prone ubiquitinated proteins by timosaponin A-III

C.N. Lok, L.K. Sy, F. Liu, C.M. Che

The Journal of Biological Chemistry, 2011, 286, 31684-31696

Abstract

Chemical modulators of autophagy provide useful pharmacological tools for examination of

autophagic processes, and also may lead to new therapeutic agents for diseases in which control of cellular sequestration and degradation capacity are beneficial. We have identified that timosaponin A-III (TAIII), a medicinal saponin reported to exhibit anticancer properties and improve brain function, is a pronounced activator of autophagy. In this work, the salient features and functional role of TAIII-induced autophagy were investigated. In TAIII-treated cells, autophagic flux with increased formation of autophagosomes and conversion into autolysosomes is induced in association with inhibition of mammalian target of rapamycin activity and elevation of cytosolic free calcium. The TAIII-induced autophagy is distinct from conventional induction by rapamycin, exhibiting large autophagic vacuoles that appear to contain significant contents of endosomal membranes and multivesicular bodies. Furthermore, TAIII stimulates biosynthesis of cholesterol, which is incorporated to the autophagic vacuole membranes. The TAIII-induced autophagic vacuoles capture ubiquitinated proteins, and in proteasome-inhibited cells TAIII promotes autophagy of aggregation-prone ubiquitinated proteins. Our studies demonstrate that TAIII induced a distinct form of autophagy, and one of its pharmacological actions is likely to enhance the cellular quality control capacity via autophagic clearance of otherwise accumulated ubiquitinated protein aggregates.

10. Tissue-Spray Ionization Mass Spectrometry for Raw Herb Analysis

S.L.F.Chan, M.Y.M. Wong, H.W. Tang, C.M. Che, K.M. Ng

Rapid Communications in Mass Spectrometry, 2011, 25, 2837-2843

Abstract

Tissue-spray ionization mass spectrometry is developed for the in situ chemical analysis of raw herbs under ambient conditions. We demonstrated that analyte molecules could be directly sprayed and ionized from solvent-wetted ginseng tissues upon the application of high electrical voltage to the tissue sample. Abundant phytochemicals/ metabolites, including ginsenosides, amino acids and oligosaccharides, could be detected from ginseng tissues when the tissue-spray experiments were conducted in positive ion mode. Thermally labile and easily hydrolyzed malonyl-ginsenosides were also detected in negative ion mode. The tissue-spray ionization method enables the direct detection of analytes from raw herb samples and preserves the sample integrity for subsequent morphological and/ or microscopic examination. In addition, this method is simple and fast for chemical profiling of wild-type and cultivated-type American ginsengs with differentiation.

11. Chemical and biological analysis of active free and conjugated bile acids in animal bile using HPLC-ELSD and MTT methods

N. Wang, Y. Feng, T.N. Xie, W. Su, M. Zhu, O. Chow, Y. Zhang, K.M. Ng, C.H. Leung, Y. Tong

Experimental and Therapeutic Medicine, 2011, 2, 125-130

Abstract

The aim of the present study was to determine the chemical composition and in vitro cytotoxic activity of seven bile samples and bile acids using the high-performance liquid chromatography (HPLC)-evaporative light scattering detector (ELSD) method. Free and conjugated bile acid standards were used to identify and quantify the chemical components of the seven animal bile samples. The MTT assay was used to determine the cytotoxic effect of the animal bile samples and the free and conjugated bile acids on hepatocellular carcinoma MHCC97-L cells. Chemical analysis revealed that the bile samples from the different animals shared little similarity in terms of their composition. A cell viability assay revealed that cattle bile, as well as its major components, DCA, CDCA and TCDCA, exhibited a marked cytotoxic effect on the hepatocellular carcinoma MHCC97-L cells. The bear bile samples that originated from the Asian black bear and the American black bear contained a unique component, TUDCA, which distinguished them from the other animal bile, though their inhibitory action on MHCC97-L cells was not markedly distinct. The present study reveals that cattle bile may be a potential alternative to bear bile for hepatocarcinoma therapy.

12. Alisol B, a novel inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump, induces autophagy, endoplasmic reticulum stress, and apoptosis

B.Y. Law, M. Wang, D.L. Ma, F. Al-Mousa, F. Michelangeli, S.H. Cheng, M.H. Ng, K.F. To, A.Y. Mok, R.Y. Ko, S.K. Lam, F. Chen, C.M. Che, P. Chiu, B.C. Ko

Molecular Cancer Therapy, 2010, 9, 718-730

Abstract

Emerging evidence suggests that autophagic modulators have therapeutic potential. This study aims to identify novel autophagic inducers from traditional Chinese medicinal herbs as potential antitumor agents. Using an image-based screen and bioactivity-guided purification, we identified alisol B 23-acetate, alisol A 24-acetate, and alisol B from the rhizome of Alisma orientale as novel inducers of autophagy, with alisol B being the most potent natural product. Across several cancer cell lines, we showed that alisol B-treated cells displayed an increase of autophagic flux and formation of autophagosomes, leading to cell cycle arrest at the G(1) phase and cell death. Alisol B induced calcium mobilization from internal stores, leading to autophagy through the activation of the CaMKK-AMPK-mammalian target of rapamycin pathway. Moreover, the disruption of calcium

homeostasis induces endoplasmic reticulum stress and unfolded protein responses in alisol B-treated cells, leading to apoptotic cell death. Finally, by computational virtual docking analysis and biochemical assays, we showed that the molecular target of alisol B is the sarcoplasmic/endoplasmic reticulum Ca2+ ATPase. This study provides detailed insights into the cytotoxic mechanism of a novel antitumor compound.

13. Structure-Based Discovery of Natural-Product-like TNF-alpha Inhibitors

D. S.H. Chan, H.M. Lee, F. Yang, C.M. Che, C. C. L. Wong, R. Abagyan, C.H. Leung,

D.L. Ma

Angewandte Chemie, International Edition, 2010, 49, 2860-2864

Abstract

We have discovered two small-mol. TNF-α inhibitors from a natural-product and natural-product-like chem. libraries using structure-based design. The identification of quinuclidine and quinolizine compds. represents, to the best of our knowledge, only the third and fourth examples of the direct targeting of TNF-α by a small mol. Importantly, indoloquinolizidine compd. was found to be more potent against TNF-α in the ELISA compared to SPD304, the strongest small-mol. TNF-α inhibitor reported to date. We are currently conducting computer-based hit-to-lead ere incubated optimization to generate addnl. analogs for in vitro centrations. testing.

14. Subcellular localization of a fluorescent artemisinin derivative to endoplasmic reticulum

Y. Liu, C.N. Lok, B.C Ko, T.Y. Shum, M.K. Wong, C.M. Che

Organic Letter, 2010,12, 1420-1423

Abstract

A cytotoxic artemisinin derivative conjugated with a fluorescent dansyl moiety was synthesized and its subcellular localization in Hep3B cells was examined. Comparison of the localization signals of the fluorescent artemisinin derivative with organelle specific dyes revealed that endoplasmic reticulum (ER) is the main site of its accumulation.

15. Inhibition of mutagenic PhIP formation by epigallocatechin gallate via scavenging of phenylacetaldehyde

K.W. Cheng, C.C. Wong, J. Chao, C. Lo, F. Chen, I.K. Chu, C.M. Che, C.T. Ho, M. Wang

Molecular Nutrition & Food Research, 2009, 53, 716-725

Abstract

Chemical model investigation showed that both epigallocatechin gallate (EGCG) and its peracetate, which has all the hydroxyl groups acetylated, effectively reduced the formation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), the most abundant mutagenic heterocyclic amine found in foods. Mechanistic study was subsequently carried out to characterize the probable inhibitory mechanism involved. GC-MS analysis showed that EGCG in only one-fourth molar quantity of phenylalanine reduced formation of phenylacetaldehyde, a key PhIP intermediate by nearly 90%. Its peracetate also showed similar inhibitory activity. This further supported the existence of an antioxidant-independent mechanism contributing to the inhibition of PhIP formation by EGCG. Subsequent LC-MS analyses of samples from a wide range of model systems consisting of PhIP precursors showed the generation of characteristic analytes with molecular weight corresponding to the sum of EGCG and phenylalanine fragment(s) only in models where phenylalanine and EGCG were simultaneously present. An isotope-labeling study revealed that these analytes all contained fragment(s) of phenylalanine origin. Direct reaction employing phenylacetaldehyde and EGCG further confirmed the capability of EGCG to form adducts with phenylacetaldehyde, thus reducing its availability for PhIP formation. Finally, an investigation of the time course of the generation of postulated adduction products supported EGCG as an effective inhibitor of PhIP formation in prolonged heating processes.

16. Hepatoprotective Effects of Coptidis Rhizoma Aqueous Extract on Carbon Tetrachloride-Induced Acute Liver Hepatotoxicity in Rats

X.Ye, Y. Feng, Y. Tong, K.M. Ng, S.W. Tsao, G.K.K. Lau, C.W. Sze, Y. Zhang, J. Tang, J.Shen, S. Kobayashi

Journal of Ethnopharmacology, 2009, 124, 130-136

Abstract

Aim of the study

Coptidisrhizoma (CR, Chinese name is Huanglian) has been used in treating infectious and inflammatory diseases for two thousand years in Traditional Chinese Medicine (TCM). Its related pharmacological basis for the therapeutics has been studied intensively, but CR can also be used for vomiting of "dampness-heat type or acid regurgitation" due to "liver-fire attacking stomach" in TCM, whose symptoms seem to link the hepatic and biliary disorders, yet details in the therapies of liver diseases and underlying mechanism(s) remain unclear. To clarify this ethnopharmacological

relevance, hepatoprotective effect of Coptidisrhizomaaqueousextract (CRAE) and its possible mechanism were studied in rats intoxicated with carbontetrachloride (CCl4) in the present study.

Materials and methods

Sprague–Dawley (SD) rats aged 7 weeks old were intraperitoneally injected with CCl4 at a dose of 1.0 ml/kg as a 50% olive oil solution. The rats were orally given the CRAE at doses of 400, 600, 800 mg/kg and 120 mg/kg berberine body weight (BW) after 6 h of CCl4 treatment. At 24 h after CCl4 injection, samples of blood and liver were collected and then biochemical parameters and histological studies were carried out.

Results

The results showed that CRAE and berberine inhibited significantly the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and increased the activity of superoxide dismutase (SOD). Observation on the hepatoprotective effect of berberine was consistent to that of CRAE.

Conclusion

The study is the first time to demonstrate that CRAE has hepatoprotective effect on acute liver injuries induced by CCl4, and the results suggest that the effect of CRAE against CCl4-induced liver damage is related to antioxidant property.

17. Bear Bile: Dilemma of Traditional Medicinal Use and Animal Protection

Y. Feng, K.Y. Siu, N. Wang, K.M. Ng, S.W. Tsao, T. Nagamatsu, Y. Tong

Journal of Ethnobiology and Ethnomedicine, 2009, 5, 1-9

Abstract

Bear bile has been used in Traditional Chinese Medicine (TCM) for thousands of years. Modern investigations showed that it has a wide range of pharmacological actions with little toxicological side effect and the pure compounds have been used for curing hepatic and biliary disorders for decades. However, extensive consumption of bear bile made bears endangered species. In the 1980's, bear farming was established in China to extract bear bile from living bears with "Free-dripping Fistula Technique". Bear farming is extremely inhumane and many bears died of illness such as chronic infections and liver cancer. Efforts are now given by non-governmental organizations, mass media and Chinese government to end bear farming ultimately. At the same time, systematic research has to be done to find an alternative for bear bile. In this review, we focused on the literature, laboratory and clinical results related to bear bile and its substitutes or alternative in English and Chinese databases. We examined the substitutes or alternative of bear bile from three aspects: pure compounds derived from bear bile, biles from other animals and herbs from TCM. We then

discussed the strategy for stopping the trading of bear bile and issues of bear bile related to potential alternative candidates, existing problems in alternative research and work to be done in the future.

18. Timosaponin A-III induces autophagy preceding mitochondria-mediated apoptosis in HeLa cancer cells

L.K. Sy, S.C. Yan, C.N. Lok, R.Y. Man, C.M. Che

Cancer Research, 2008, 68, 10229-10237

Abstract

Timosaponin A-III (TAIII), a saponin isolated from the rhizome of Anemarrhena asphodeloides, exhibits potent cytotoxicity and has the potential to be developed as an anticancer agent. Here, we provide evidence that TAIII induces autophagy in HeLa cells followed by apoptotic cell death. TAIII-induced autophagy was morphologically characterized by the formation of membrane-bound autophagic vacuoles recognizable at the ultrastructural level. TAIII-treated cells expressing green fluorescent protein (GFP)-labeled microtubule-associated protein 1 light chain 3 (LC3) displayed punctate fluorescence indicative of LC3 recruitment to the autophagosome. This was associated with the conversion of LC3-I (the cytosolic form) into LC3-II (the lipidated form located on the autophagosome membrane). TAIII treatment also induced mitochondrial dysfunction involving overproduction of reactive oxygen species and reduction of mitochondrial membrane potential accompanied by induction of mitochondrial permeability transition. Prolonged exposure to TAIII resulted in cytochrome c release and caspase-3 activation, events that signified the onset of apoptotic cell death. TAIII-induced autophagy preceded apoptosis, as evidenced by early autophagic vacuole formation, GFP-LC3 translocation, and LC3-II increase in the absence of caspase-3 cleavage. Notably, TAIII-mediated apoptotic cell death was potentiated by treatment with autophagy inhibitor 3-methyladenine or small interfering RNA against the autophagic gene beclin 1. These findings suggest that TAIII-elicited autophagic response plays a protective role that impedes the eventual cell death. In terms of structure-activity relationship, the sugar chain in TAIII is indispensable to the drug action, as the sugar-lacking aglycone sarsasapogenin did not induce autophagy and exhibited weaker cytotoxicity.

19. Proteomic and transcriptomic study on the action of a cytotoxic saponin (Polyphyllin D): induction of endoplasmic reticulum stress and mitochondria-mediated apoptotic pathways

F.M. Siu, D.L. Ma, Y.W. Cheung, C.N. Lok, K. Yan, Z. Yang, M. Yang, S. Xu, B.C. Ko, Q.Y. He, C.M. Che

Proteomics, 2008, 8, 3105-17

Abstract

Polyphyllin D (PD) is a potent cytotoxic saponin found in Paris polyphylla. In the present study, bioinformatic, proteomic and transcriptomic analyses were performed to study the mechanisms of action of PD on human nonsmall cell lung cancer (NSCLC) cell line (NCI-H460). Using a gene expression-based bioinformatic tool (connectivity map), PD was identified as a potential ER stress inducer. Our proteomic and transcriptomic analyses revealed that PD treatment led to upregulation of typical ER stress-related proteins/genes including glucose-regulated protein 78 (BiP/GRP78) and protein disulfide isomerase (PDI). In particular, elevated expression of C/EBP homologous transcription factor (chop) and activation of caspase-4 occurred at early time point (8 h) of PD treatment, signifying an initial ER stress-mediated apoptosis. Induction of tumor suppressor p53, disruption of mitochondrial membrane, activation of caspase-9 and caspase-3 were detected upon prolonged PD treatment. Collectively, these data revealed that PD induced the cytotoxic effect through a mechanism initiated by ER stress followed by mitochondrial apoptotic pathway. The ability of activating two major pathways of apoptosis makes PD an attractive drug lead for anticancer therapeutics.

20. Transition-metal-catalyzed group transfer reactions for selective C-H bond functionalization of artemisinin

Y. Liu, W. Xiao, M.K. Wong, C.M. Che

Organic Letter, 2007, 9, 4107-4110

Abstract

Three types of novel artemisinin derivatives have been synthesized through transition-metal-catalyzed intramolecular carbenoid and nitrenoid C-H bond insertion reactions. With rhodium complexes as catalysts, lactone 11 was synthesized via carbene insertion reaction at the C16 position in 90% yield; oxazolidinone 13 was synthesized via nitrene insertion reaction at the C10 position in 87% yield based on 77% conversion; and sulfamidate 14 was synthesized via nitrene insertion reaction at the C8 position in 87% yield.

21. Dioscin (saponin)-induced generation of reactive oxygen species through mitochondria dysfunction: a proteomic-based study

Y. Wang, C.M .Che, J.F. Chiu, Q.Y. He

Journal of Proteome Research, 2007, 6, 4703-4710

Abstract

It is generally believed that traditional Chinese medicine such as saponins has great value as potent cancer prevention and chemotherapeutic agents; however, the molecular basis for their activities is for the most part lacking. In the present study, we used proteomics to examine the cytotoxic effect of dioscin, a glucoside saponin, on human myeloblast leukemia HL-60 cells. Dioscin induced apoptosis in HL-60 cells in a time-dependent manner. Protein profiling of the microsomal fraction with enriched plasma membrane proteins isolated from HL-60 cells revealed that proteins act as chaperones and/or mediators of protein folding and were substantially altered in expression cells upon dioscin stimuli. Further biochemical study indicated that mitochondria dysfunction caused generation of reactive oxygen species (ROS), leading to the changes in protein expression. The mitochondrial transmembrane potential (DeltaPsi m) inhibitor aristolochic acid (ArA) partially abrogated the dioscin-initiated death receptor apoptosis pathway and cell death. The current study provided detailed evidence to support that dioscin is capable of inducing apoptosis in mammalian cells, in which the mitochondria-initiated apoptosis pathway plays an important role.

22. In vivo analysis and spatial profiling of phytochemicals in herbal tissue by matrix-assisted laser desorption/ionization mass spectrometry

K.M. Ng, Z. Liang, W. Lu, H.W. Tang, Z. Zhao, C.M. Che, Y.C. Cheng

Analytical Chemistry, 2007, 79, 2745-2755

Abstract

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) was developed for spatial profiling of phytochemicals and secondary metabolites in integrated herbal tissue without solvent extraction. Abundant alkaloid ions, including (+)-menisperine (m/z 356), magnoflorine (m/z 342), stepharanine (m/z 324), protonated sinomenine (m/z 330), protonated sinomendine (m/z 338), and a metabolite at m/z 314, could be directly desorbed from alpha-cyano-4-hydroxycinnamic acid- (CHCA-) coated stem tissue of Sinomenium acutum upon N2 laser (337 nm) ablation, while the ion signals desorbed from sinapinic acid- (SA-) coated and 2,5-dihydroxybenzoic acid- (DHB-) coated stem tissue were at least 10 times weaker. Solvent composition in the matrix solution could have significant effects on the ion intensity of the metabolites. Under optimized conditions that maximize the ion intensity and form homogeneous matrix crystals on the tissue surface, spatial distributions of the metabolites localized in different tissue regions, including cortex, phloem, xylem, rim, and pith, and their relative abundances could be semiquantitatively determined. The three metabolites

detected at m/z 356, 342, and 314 showed specific distributions in the herbal samples collected from different growing areas, while others were not. By applying principal component analysis (PCA), the characteristic metabolites in specific tissue regions could be easily determined, allowing unambiguous differentiation of the herbal samples from different geographic locations.

23. Genome-wide Biological Response Fingerprinting (BioReF) of the Chinese Botanical Formulation ISF-1 Enables the Selection of Multiple Marker Genes as a Potential Metric for Quality Control

J. Rong, R. Tilton, J. Shen, K.M. Ng, C. Liu, P.K.H. Tam, A.S.Y. Lau, Y.C. Cheng

Journal of Ethnopharmacology, 2007, 113, 35 – 44

Abstract

Quality control plays a critical role in the process of translating the traditional/alternative medicines into modern evidence-based therapies. High performance liquid chromatography (HPLC) is widely applied to assess the chemical composition of botanical drug products. The chromatographic fingerprints or chemical profiles are currently used as the de facto quality control metric. As a complement to chemical profiles, a biological quality control assessment offers distinct advantages. This study describes a genome-wide biological response fingerprinting (BioReF) approach to define a set of marker genes that define a signature pattern for a specific botanical formulation. These marker genes are chosen on the basis of the levels of the regulated expression and the involvement in the cellular signaling pathways. Subsequently, qRT-PCR technique is used to simultaneously monitor the gene expression of multiple marker genes in an efficient and quantitative manner. This set of marker genes represents the biological responses of human cells to the chemical composition of the botanical drug that could serve as potential quality control of botanical drugs in terms of the consistency of biological activities. We demonstrate the BioReF approach with a well-documented Chinese Medicine formula, designated as ISF-1, traditionally used for the management of post-stroke disorders. A set of nine marker genes were selected to assess the batch-to-batch consistency of the biological effects of ISF-1. This approach provides a potential comprehensive and cost-effective quality control metric of the biological activities of botanical drugs.

24. In vivo transformations of artemisinic acid in Artemisia annua plants

L.K. Sy, G. D. Brown

Tetrahedron, 2007, 63, 9548-9566

Abstract

Artemisinic acid labeled with both C-13 and H-2 at the 15-position has been fed to intact plants of Artemisia annua via the cut stem, and its in vivo transformations studied by 1D- and 2D-NMR spectroscopy. Seven labeled metabolites have been isolated, all of which are known as natural products from this species. The transformations of artemisinic acid-as observed both for a group of

plants, which was kept alive by hydroponic administration of water and for a group, which was allowed to die by desiccation-closely paralleled those, which have been recently described for its 11,13-dihydro analog, dihydroartemisinic acid. It seems likely therefore that similar mechanisms, involving spontaneous autoxidation of the Delta(4,5) double bond in both artemisinic acid and dihydroartemisinic acid and subsequent rearrangements of the resultant allylic hydroperoxides, may be involved in the biological transformations, which are undergone by both compounds. All of the sesquiterpene metabolites, which were obtained from in vivo transformations of artemisinic acid retained their unsaturation at the 11,13-position, and there was no evidence for conversion into any 11,13-dihydro metabolite, including artemisinin, the antimalarial drug, which is produced by A. annua. This observation led to the proposal of a unified biosynthetic scheme, which accounts for the biogenesis of many of the amorphane and cadinane sesquiterpenes that have been isolated as natural products from A. annua. In this scheme, there is a bifurcation in the biosynthetic pathway starting from amorpha-4,11-diene leading to either artemisinic acid or dihydroartemisinic acid; these two committed precursors are then, respectively, the parents for the two large families of highly oxygenated 11,13-dehydro and 11,13-dihydro sesquiterpene metabolites, which are known from this species.

25. In vivo transformations of dihydro-epi-deoxyarteannuin B in Artemisia annua plants

L.K. Sy, G. D. Brown

Tetrahedron, 2007, 63, 9536-9547

Abstract

15-(CH3)-C-13-H-2-dihydro-epi-deoxyarteannuin B (4a) has been fed to intact Artemisia annua plants via the root and three labeled metabolites (17a-19a) have been identified by 1D- and 2D-NMR spectroscopies. The in vivo transformations of 4a in A. annua are proposed to involve enzymatically-mediated processes in addition to possible spontaneous autoxidation. In the hypothetical spontaneous autoxidation pathway, the tri-substituted double bond in 4a appears to have undergone 'ene-type' reaction with oxygen to form an allylic hydroperoxide, which subsequently rearranges to the allylic hydroxyl group in the metabolite 3 alpha-hydroxy-dihydro-epi-deoxyarteannuin B (17a). In the enzymatically-mediated pathways, compound 17a has then been converted to its acetyl derivative, 3 alpha-acetoxy-dihydro-epi-deoxyarteannuin B (18a), while oxidation of 4a at the 'unactivated' 9-position has yielded 9 beta-hydroxy-dihydro-epi-deoxyarteannuin B (19a). Although all of the natural products artemisinin 1), arteannuin K 7), arteannuin L 8), and arteannuin M 9) have been suggested previously as hypothetical metabolites from dihydro-epi-deoxyarteannuin B in A. annua, none were isolated in labeled form in this study. It is argued that the nature of the transformations undergone by compound 4a are more consistent with a degradative metabolism, designed to eliminate this compound from

the plant, rather than with a role as a late precursor in the biosynthesis of artemisinin or other natural products from A. annua.

26. Proteomic approach to study the cytotoxicity of dioscin (saponin)

Y. Wang, Y.H. Cheung, Z. Yang, J.F. Chiu, C.M. Che, Q.Y. He

Proteomics, 2006, 6, 2422-2432

Abstract

Dioscin, extracted from the root of Polygonatum zanlanscianense pamp, exhibits cytotoxicity towards human myeloblast leukemia HL-60 cells. Proteomic analysis revealed that the expression of mitochondrial associated proteins was substantially altered in HL-60 cells corresponding to the dioscin treatment, suggesting that mitochondria are the major cellular target of dioscin. Mitochondrial functional studies validated that mitochondrial apoptotic pathway was initiated by dioscin treatment. Changes in proteome other than mitochondrial related proteins implicate that other mechanisms were also involved in dioscin-induced apoptosis in HL-60 cells, including the activity impairment in protein synthesis, alterations of phosphatases in cell signaling, and deregulation of oxidative stress and cell proliferation. Current study of protein alterations in dioscin-treated HL-60 cells suggested that dioscin exerts cytotoxicity through multiple apoptosis-inducing pathways.

27. Analytical application of acetate anion in negative electrospray ionization mass spectrometry for the analysis of triterpenoid saponins--ginsenosides

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Rapid Communications in Mass Spectrometry, 2006, 20, 1545-1550

Abstract

Ginsenosides containing different numbers of glycosyl groups can be easily differentiated based on the formation of characteristic ginsenoside-acetate adduct anions and deprotonated ginsenosides generated by electrospray ionization (ESI) of methanolic solutions of ginsenosides (M) and ammonium acetate (NH_4OAc). Ginsenosides containing two glycosyl groups gave a characteristic mass spectral pattern consisting of $[M+2OAc]^{2-}$, $[M-H+OAc]^{2-}$ and $[M-2H]^{2-}$ ions with m/z values differing by 30 Th, while this mass spectral pattern was not observed for ginsenosides containing one glycosyl group. Formation of $[M+2OAc]^{2-}$ was influenced by the chain length of glycosyl groups and was used to differentiate the ginsenosides containing different combinations of monosaccharide and disaccharide units in the glycosyl groups. Under identical collisional activation conditions, $[M+OAc]^-$, $[M-H+OAc]^{2-}$ and $[M+2OAc]^{2-}$ underwent proton abstractions predominantly to generate $[M-H]^-$, $[M-2H]^{2-}$ and $[M-H+OAc]^{2-}$ ions, respectively. The ion intensity ratios, I[M-H](-/I) $[M+OAc]^-$, I[M-2H](2-/I) $[M-H+2OAc]^{2-}$ and I[M-H+OAc](2-/I) $[M+OAc]^{2-}$, being sensitive to the structural differences of ginsenosides, could differentiate the isomeric ginsenosides, including (i) Rf, F11 and

Rg1, (ii) Rd and Re, and (iii) Rb2 and Rc. Additionally, NH_4OAc was found to enhance the sensitivity of detection of ginsenosides in the form of [M-H]- down to the femtomole level.

28. Neuroprotective effects of anti-aging oriental medicine Lycium barbarum against beta-amyloid peptide neurotoxicity

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Experimental Gerontology, 2005, 40, 716-727

Abstract

As aged population dramatically increases in these decades, efforts should be made on the intervention for curing age-associated neurodegenerative diseases such as Alzheimer's disease (AD). Natural plant extracts of Lycium barbarum are well-known to exhibit anti-aging effects. We therefore hypothesized that they exhibit neuroprotective effects against toxins in aging-related neurodegenerative diseases. In this study, we aimed to investigate whether extracts from L. barbarum have neuroprotective effects against toxicity of fibrillar Abeta(1-42) and Abeta(25-35) fragments. Primary rat cortical neurons exposed to Abeta peptides resulted in apoptosis and necrosis. Pre-treatment with extract isolated from L. barbarum significantly reduced the release of lactate dehydrogenase (LDH). In addition, it attenuated Abeta peptide-activated caspases-3-like activity. The extract elicited a typical dose-dependent neuroprotective effect. Effective dosage of this extract was wider than that of a well-known western neuroprotective medicine lithium chloride (LiCl). We have further examined the underlying mechanisms of the neuroprotective effects. In agreement with other laboratories, Abeta peptides induce a rapid activation of c-Jun N-terminal kinase (JNK) by phosphorylation. Pre-treatment of aqueous extract markedly reduced the phosphorylation of JNK-1 (Thr183/Tyr185) and its substrates c-Jun-I (Ser 73) and c-Jun-II (Ser 63). Taken together, we have proved our hypothesis by showing neuroprotective effects of the extract from L. barbarum. Study on anti-aging herbal medicine like L. barbarum may open a new therapeutic window for the prevention of AD.

29. Fluorophore-appended steroidal saponin (dioscin and polyphyllin D) derivatives

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Organic Letter, 2005, 7,669-672

Abstract

The synthesis of three fluorophore-appended derivatives of dioscin and polyphyllin D is reported herein. Starting from trillin, dansyl derivatives A-C were prepared in overall yields of 7-12% over 7-10 steps. A study of their behavior in a variety of polar solvents suggests that dansyl derivatives A-C are capable of micellar self-assembly and can maintain cytotoxicities (IC_{50} = 15-18 μM) against the HeLa carcinoma cell line evaluated by standard MTT assay.

30. Synthesis and cytotoxicity studies of artemisinin derivatives containing lipophilic alkyl carbon chains

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Organic Letter, 2005, 7, 1561-1564

Abstract

[reaction: see text] Cytotoxic artemisinin derivatives have been synthesized by a modular approach of "artemisinin + linker + lipophilic alkyl carbon chain". A strong correlation between the length of the carbon chains and the cytotoxicities against human hepatocellular carcinoma (HepG2) was revealed. Notably, compared with artemisinin (IC_{50} = 97 μM), up to 200-fold more potent cytotoxicity (IC_{50} = 0.46 μM) could be achieved by attachment of a C(14)H(29) carbon chain to artemisinin via an amide linker.