

ISSN 1881-7831 Online ISSN 1881-784X

# DD & T

## Drug Discoveries & Therapeutics

Volume 6, Supplement  
October, 2012

### ABSTRACTS

Japan-China Joint Medical Workshop 2012  
Sanjo Conference Hall, The University of Tokyo  
October 22-24, Tokyo, Japan



[www.ddtjournal.com](http://www.ddtjournal.com)



# **Japan-China Joint Medical Workshop 2012 on Drug Discoveries and Therapeutics**

## **New Perspectives in Cancer Prevention, Diagnosis, Treatments, and Drug Discoveries**

Sanjo Conference Hall, The University of Tokyo  
October 22-24, Tokyo, Japan

### **ABSTRACTS**



[www.biosciencetrends.com](http://www.biosciencetrends.com)  
[www.ddtjournal.com](http://www.ddtjournal.com)  
[www.irdrjournal.com](http://www.irdrjournal.com)

# IRCA-BSSA Group

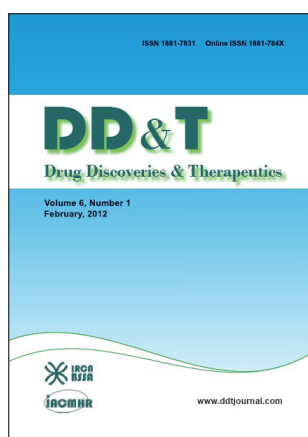
## Center for Medical Standards Research

Center for Medical Standards Research (CMSR), established by IRCA-BSSA Group, is an international non-profit independent organization. It aims to provide more scientific and objective medical evidence for medical personnel by promoting scientific research as well as advocating large-scale multi-center prospective studies. CMSR also actively promotes and compiles medical knowledge in multiple fields via clinical pathway studies. It seeks to clearly indicate the personal knowledge and accumulated experience of medical experts and promotes evidence-based medicine, standardized management of the treatment process, "treatment guidelines" for individual diseases and systematic cataloging of research findings in related disciplines. With the support of the IRCA-BSSA Group Journals, the ultimate goals of CMSR are to build a global platform for the exchange of information on standardized management of care and assessment of its effectiveness in Asia and to continue promoting international studies and cooperation in medical and health fields.

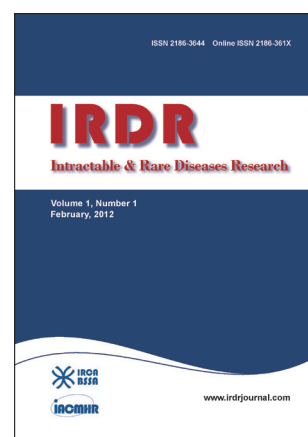
### IRCA-BSSA Group Journals



ISSN: 1881-7815  
Online ISSN: 1881-7823  
CODEN: BTIRCZ  
Issues/Year: 6  
Language: English  
Publisher: IACMHR Co., Ltd.  
[www.biosciencetrends.com](http://www.biosciencetrends.com)



ISSN: 1881-7831  
Online ISSN: 1881-784X  
CODEN: DDTRBX  
Issues/Year: 6  
Language: English  
Publisher: IACMHR Co., Ltd.  
[www.ddtjournal.com](http://www.ddtjournal.com)



ISSN: 2186-3644  
Online ISSN: 2186-361X  
CODEN: IRDRA3  
Issues/Year: 4  
Language: English  
Publisher: IACMHR Co., Ltd.  
[www.irdjournal.com](http://www.irdjournal.com)

# Contents

## Lectures

### Session I Cancer diagnosis and cancer treatment

- 1 **Surgical treatment for gastric cancer in China**  
*Xishan Hao*
- 3 **Safety validation of different decision trees in patients with liver tumor using fifty-fifty criteria**  
*Xianqiang Kai, Jiangying Luo, Wenping Lv, Jiahong Dong*
- 4 **Liver transplantation for hepatocellular carcinoma: Japanese experience**  
*Sumihito Tamura, Yasuhiko Sugawara, Noriyo Yamashiki, Kayo Nojiri, Junichi Kaneko, Taku Aoki, Yoshihiro Sakamoto, Kiyoshi Hasegawa, Wei Tang, Norihiro Kokudo*
- 5 **Evidence-based clinical practice guidelines: Standardized management for hepatocellular carcinoma**  
*Peipei Song, Wei Tang, Norihiro Kokudo*
- 6 **Transarterial chemoembolization (TACE) combined molecular targeted therapy of hepatocellular carcinoma**  
*Wentao Li, Zheng Yuan, Weijun Peng*
- 8 **Clinical research on intracavitary treatment with Cinobufacini injection for malignant pleural or peritoneal effusions**  
*Chao An, Qin Zhou, Minghuan Zuo, Quanwang Li, Min Jiang, Tao Sun, Li Xiao, Zhanbing Qiao, Kaiwen Hu*
- 9 **The prediction and treatment strategy for hepatocellular carcinoma in high risk recurrence after hepatectomy**  
*Tianqiang Song*

### Session II Cancer prevention and drug discoveries

- 10 **Towards quantitative endogenous network theory on cancer genesis and progression: Beyond “cancer as diseases of genome”**  
*P. Ao, D. Galas, L. Hood, X.M. Zhu*
- 11 **Study of Na<sup>+</sup>/K<sup>+</sup>-ATPase as a therapeutic target of product that contain cardiacglycosides**  
*Litao Xu, Zhiqiang Meng*

- 12 **Design and prepare theranostic nano-vectors**  
*Na Zhang*
- 13 **STAT3 as a target for HCC therapy**  
*Xiaoxia Sun, Qiangjun Sui*
- 14 **Discovery of anti-cancer chemical constituents from the Chinese bryophytes**  
*Hongxiang Lou*
- 15 **Diterpenoids with anti-cancer potential from Chinese medicinal plants**  
*Xiaoning Wang, Bo Wang, Hongxiang Lou*

## Oral presentation

### Session I Cancer treatment and cancer diagnosis

- 16 **Real-time identification of pancreatic leak following pancreatic resection using fluorescent probes activated by chymotrypsin and  $\gamma$ -glutamyltranspeptidase**  
*Suguru Yamashita, Takeaki Ishizawa, Masayo Sakabe, Junichi Kaneko, Sumihito Tamura, Yoshihiro Sakamoto, Taku Aoki, Yasuhiko Sugawara, Kiyoshi Hasegawa, Yasuteru Urano, Norihiro Kokudo*
- 17 **Validation of a preoperative predictive scoring system for postoperative pancreatic fistula after pancreaticoduodenectomy**  
*Ti Zhang, Huikai Li, Qiang Li*
- 18 **Laparoscopic radio frequency ablation for hepatocellular carcinoma of specific location**  
*Yuesi Zhong, Meihai Deng, Heping Fang, Kai Li, Ruiyun Xu*
- 20 **ICG-fluorescent imaging during liver surgery for evaluation of portal uptake function in veno-occlusive regions**  
*Yoshikuni Kawaguchi, Takeaki Ishizawa, Yoichi Miyata, Suguru Yamashita, Koichi Masuda, Shoichi Satou, Sumihito Tamura, Junichi Kaneko, Yoshihiro Sakamoto, Taku Aoki, Kiyoshi Hasegawa, Yasuhiko Sugawara, Norihiro Kokudo*
- 21 **Risk factors for the postoperative hemorrhage of digestive tract in patients with malignant obstructive jaundice**  
*Dalu Kong, Qiang Li, Xishan Hao*
- 22 **Intraperitoneal injection of cinobufatacini and cancerous ascites treated by double-pathway chemotherapy**  
*Yinyin Li, Yongping Wang, Xiumin Yuan, Qiang Liu, Dongping Liu*

- 23 **Hepatoblastoma in adult age: A report of 15 cases and review of literature**

*Qiang Wu*

**Session II Drug Discoveries: therapeutic targets and pre-clinical study**

- 24 **Acetylated Hsp90 as a target for cancer therapy and drug development**

*Qiuyuan Peng, Bao Yong, Zhaohu Lin, Yonghua Yang*

- 25 **KL-6 mucin in hepatobiliary and pancreatic tumors**

*Yoshinori Inagaki, Wei Tang, Kiyoshi Hasegawa, Yasuhiko Sugawara, Norihiro Kokudo*

- 26 **Identification of c-Met as a potential therapeutic target for hepatocellular carcinoma**

*Jianjun Gao, Yoshinori Inagaki, Peipei Song, Wei Tang*

- 27 **Design, synthesis and biological evaluation of 1, 2, 4, 7-substituted indole derivatives targeting serine/threonine kinase Akt/PKB**

*Peng Wang, Jianzhen Liu, Guisen Zhao*

- 28 **Usefulness of silkworm disease model for drug development**

*Kazuhisa Sekimizu*

- 29 **Identification of novel hepatitis C virus NS3 inhibitors: Psammaplin A and Halisulfate 3**

*Kazi Abdus Sala, Atsushi Furuta, Naohiro Noda, Satoshi Tsuneda, Yuji Sekiguchi, Atsuya Yamashita, Kohji Moriishi, Masamichi Nakakoshi, Masayoshi Tsubuki, Hidenori Tani, Junichi Tanaka, Nobuyoshi Akimitsu*

- 30 **Synthesis of dihydroxypyrimidine-4-carboxamide derivatives as HIV-1 integrase inhibitors**

*Shenghui Yu, Nouri Neamati, Guisen Zhao*

- 31 **The multifunctional nanoscaled delivery system for codelivery of nucleic acids and chemotherapeutics**

*Chunxi Liu, Na Zhang*

- 32 **13F-1, a peptidomimetics compound inhibited Colo205 cancer growth by targeting APN (CD13)**

*Houli Zhang, Shuxiang Cui*

- 33 **Identifying and optimizing novel B-RafV600E inhibitors by virtual screening**

*Zhijian Xu, Guirui Yan, Heyao Wang, Weiliang Zhu*

- 34 **Study on the penetration and inhibition of angiogenesis of endostatin with the mediation of Tat PTD**

*Xinke Zhang, Yan Li, Haining Tan, Yanna Cheng, Fengshan Wang*

- 35 **Novel and potent HIV-1 NNRTIs research: design, synthesis and biological evaluation**

- Xinyong Liu, Peng Zhan, Xuwang Chen, Wenmin Chen, Ye Tian, Xiao Li, Christophe Pannecouque, Jan Balzarini, Erik De Clercq*
- 36 **Recombinant HDL nanoparticles for drug delivery and imaging of the liver**  
*Yuhong Xu, Mengjie Rui*
- 37 **Effects of monoclonal antibodies, MLS128 against Tn-antigen and 1H7 against insulin-like growth factor-I receptor, on colon cancer cell growth**  
*Normaiza Zamri, Naoya Masuda, Fumie Oura, Yukiko Yajima, Hiroshi Nakada, Yoko Fujita-Yamaguchi*
- 38 **6r, a novel oxadiazole analogue of ethacrynic acid, inhibits proliferation of human cancer cells *in vitro* and tumour xenografts *in vivo* by induction of cell apoptosis**  
*Zhang Peng, Jinhua Chen, Yanna Cheng, Guisen Zhao, Xiuli Guo*
- 39 **A potential host-directed therapeutic target against HCV infection elucidated by RNA interference screen**  
*Ri Sho, Xuhong Zhang, Hisayoshi Watanabe, Takafumi Saito, Rika Ishii, Yoshiyuki Ueno, Seiji Hongo, Akira Fukao*
- 40 **Association of tumor suppressor *p53* gene Arg72Pro polymorphism with lung cancer and control subjects in Bangladeshi population**  
*Md. Aftab Uddin*
- 41 **Design, synthesis and biological evaluation of thiazolidine derivatives as potential anti-tumor agents**  
*Hao Fang, Guizhi Xiao, Shaolei Wu, Yutao Wang, Gang Wang*
- 42 **Fluorescent probes for  $\alpha$ 1-adrenergic receptor imaging**  
*Wenhua Li, Lupei Du, Minyong Li*

### **Session III Drug Discoveries: natural bioactive agents and drug evaluation**

- 43 **Anti-hepatitis B virus activities of cinobutacini and related compounds in HepG2.2.15 cells**  
*Jufeng Xia, Xiaoyan Cui, Yoshinori Inagaki, Norihiro Kokudo, Bo Gao, Wei Tang*
- 44 **Bufalin and cinobufagin induce apoptosis in HepG2 cells through Fas- and mitochondria-mediated pathways**  
*Fanghua Qi, Yoshinori Inagaki, Norihiro Kokudo, Anyuan Li, Wei Tang*
- 45 **Use of silkworm infection model to identify novel therapeutically effective antibiotic, kaikosin E**  
*Atmika Paudel, Hiroshi Hamamoto, Makoto Urai, Jie Su, Keiko Kataoka, Kazuhisa Sekimizu*



- 46 **Identification and expression analysis of key enzymes of the terpenoids biosynthesis pathway of a liverwort *Plagiochasma appendiculatum* by EST analysis**  
*Aixia Cheng, Lei Wang, Yi Sun, Hongxiang Lou*
- 47 **Separation and absolute configuration assignment of two diastereomeric pairs of enantiomers from *Lobelia chinensis* by high-performance liquid chromatography with a circular dichroism detector**  
*Shuang Yang, Dongmei Ren*
- 48 **Antimicrobial mechanism of a novel compound, Kaikosin E**  
*Jie Su, Hiroshi Hamamoto, Atimika Paudel, Makoto Urai, Keiko Kataoka, Kazuhisa Sekimizu*
- 49 **Antiproliferative and antimicrobial activities of alkylbenzoquinone derivatives from *Ardisia kivuensis***  
*Dzoyem J. Paul, Ndontsa B. Laure, Santosh K. Guru, Hamamoto H., Matsumoto Y, Inshad A. Khan, Saxena K. Ajit, Ram A. Vishwakarma, Sekimizu K, Tane Pierre*
- 50 **Bioactive chemicals from *Curcuma xanthorrhiza***  
*Peihong Fan, Chunmei Zhang*
- 51 **Antimetastatic and antiangiogenic activities of sulfated polysaccharide of *Sepiella maindroni* ink**  
*Aizhen Zong, Ting Zhao, Yan Zhang, Xinlei Song, Yikang Shi, Hongzhi Cao, Chunhui Liu, Xianjun Qu, Fengshan Wang*

## Poster presentation

### Session I Cancer treatment and drug discoveries

- 52 **The multifunctional nanoscaled delivery system for codelivery of nucleic acids and chemotherapeutics**  
*Chunxi Liu, Na Zhang*
- 53 **Effects of anti-insulin-like growth factor-I receptor (IGF-IR) antibodies with or without the ER-retention signal KDEL on cancer cell growth**  
*Yuming Guo, Noriyuki Yuasa, Yoko Fujita-Yamaguchi*
- 54 **Chitosan-based intelligent charge-reversal system for high efficiency delivery of paclitaxel**  
*Yufeng Ma, Piping Lv, Zhanguo Yue, Wei Wei, Guanghui Ma, Rong Yu*
- 55 **Chemoprevention of intestinal adenomatous polyposis by acetyl-11-keto-beta-**

- boswellic acid (AKBA) in *APC<sup>Min/+</sup>* mice**  
*Huiping Liu, Zuhua Gao, Shuxiang Cui, Yan Wang, Baoying Li, Hongxiang Lou, Xianjun Qu*
- 56 **Inhibition of peanut agglutinin lectin-induced cancer cell growth by anti-Tn monoclonal antibody MLS128**  
*Keito Iijima, Fumie Oura, Yukiko Yajima, Hiroshi Nakada, Yoko Fujita-Yamaguchi*
- 57 **Refolding of scFv antibodies from intrabodies expressed in *E. coli* using lauroyl-L-glutamate**  
*Hiroyuki Kato, Takahito Fukuda, Hajime Sato, Noriyuki Yuasa, Yoko Fujita-Yamaguchi*
- 58 **Riccardin D-26 induces apoptosis in KB and the MDR counterpart KB/VCR cells: *In vitro* and *in vivo* studies**  
*Bin Yue, Cuirong Zhao, Huimin Xu, Yuanyuan Li, Hanni Ke, Yi Yuan, Ruiqi Wang, Hongxiang Lou, Xianjun Qu*
- 59 **Screening anti-angiogenic activities of Sanguisorba saponins by CAM model**  
*Sanhai Qin, Jun Li, Ling Zhou*
- 60 **Inhibitory effect of CMC on human chronic myeloid leukemia K562 cells and molecular mechanism underlying CMC-induced apoptosis**  
*Liyang Song, Tian Tian, Qin Zheng, Xianjing Hu, Rongmin Yu*
- 61 **Renal toxicity studies of *Clematis terniflora* DC. var. *mandshurica* on rats**  
*Yuji Liang, Zhili Hu, Jie Li, Yan Wang, Rui Zheng*
- 62 **Tumor growth suppression that makes GEF-1/Hgs a molecular target**  
*Kiyoshi Ogura, Yuko Niino, Reiko Minamikawa-Tachino*
- 63 **Effects of domain shuffling in VH-CDR3 between MLS128 and 83D4 on their Tn-antigen specificities**  
*Hajime Sato, Atsufumi Nakazawa, Yoshihiro Kitabara, Misato Maeda, Akiho Shimura, Noriyuki Yuasa, Yoshiki Yamaguchi, Yoko Fujita-Yamaguchi*
- 64 **Cancer cell migration and distribution in reconstructed basal membrane-stromal tissues with collagen gels**  
*Yo Kamoshida, Mariko Muto, Shuya Mori, Mito Yoshida, Keita Yoshimura, Taisuke Kurokawa, Chihiro Fukayama, Kyohei Fukuda, Wei Tang, Yoko Fujita-Yamaguchi, Munehiro Nakata*
- 65 **Methylamine irisolidone, a novel compound, prevents hypoxia-induced apoptosis through maintaining mitochondrial membrane potential in cardiomyocytes**  
*Yanling Mu, Fengling Wang, Ling Zhou, Ju Wang, Yanying Xie*

## Lectures

### Session I Cancer diagnosis and cancer treatment

#### Surgical Treatment for Gastric Cancer in China

Xishan Hao

*President of Chinese Anti-Cancer Association (ACA); Board of Directors, International Union Against Cancer (UICC)*

**Background and objectives:** Gastric cancer is one of the most frequent cancers in the world. In China, it is one of the top 10 cancers. The largest gastric cancer populations are from China. The purposes of this lecture were to elaborate the incidence and mortality of gastric cancer in China, to introduce our experience in gastric cancer treatment, and to discuss on reconstruction after total gastrectomy.

**Methods:** Literature review and case summary.

**Results:** There were 464,439 patients suffering gastric cancer in China 2008, accounting for 46.98% of the world's gastric cancer populations. Due to the dramatic improvements in cancer prevention and control strategy, in the recent decades, the incidence and mortality of gastric cancer have declined markedly in China. From 1981-2002 the incidence of gastric cancer in male decreased by 3.55% per year averagely in China, while the incidence in female decreased by 3.48% per year.

Between January 2003 and August 2011, a total of 3,544 patients with gastric cancer underwent surgery at Tianjin Medical University Cancer Hospital. Nearly half of them 1461 patients (41.23%) had a primary tumor located in the lower third of stomach, The largest portion of histological classification was poor differentiated adenocarcinoma (1510, accounting for 42.61%). 1977 patients (55.49%) were classified as TNM stage III.

2993 patients (84.5%) accepted curative surgery, while 551 patients accepted palliative surgery. From 2003 to 2006, 1264 patients with primary gastric cancer underwent gastric resection. For all patients, the Overall 5-year survival rate was 47%. According to UICC 7th stage, the overall 5-year survival rates were Stage I, 88%; Stage II, 65%; Stage III, 36%; and Stage IV, 20%.

Basically, digestive tract reconstruction following total gastrectomy can be classified into four types: 1. No Pouch, No Duodenal Passage, 2. Pouch without Duodenal Passage, 3. Duodenal Passage without Pouch, 4. Duodenal Passage with Pouch. Our experimental studies found that: 1. functional jejunal interposition (FJI) exerts a reservoir function to benefit food storage; 2. ICC is gastrointestinal pacemaker cell. Destroyed ICC network may have influence on intestinal motility, and less loss of ICC may contribute to preserve intestinal functional motility. Our clinical observation found that: 1. the postoperative time to food intake in the group of Jejunum untransected such as Braun, m Braun I, m Braun II, and FJI is much shorter than the group of Jejunum transected such as 'P' Roux-en-Y and 'P' jejunal interposition; 2. FJI reconstruction has the characteristics of Ligation replaces transection, Less stumps and anastomoses, Lower risk of complication, Less time-consuming, Less costly, and can reduce postoperative complications such as reflux esophagitis, Dumping Syndrome, Anemia and Roux-en-Y Syndrome.

**Conclusion:** In China, gastric cancer represents one of the most commonly diagnosed tumors; 2. D2 is a standard treatment for gastric cancer, is one of the treatment strategies to local advanced gastric cancer; 3. The 7th UICC N stage appears to provide with a reliable prognostic category for patients with gastric

cancer 4. The animal study and clinical observation indicates that FJI (Hao's) has potential application to improve the quality of life after total gastrectomy. 5. The more investigational studies are needed.

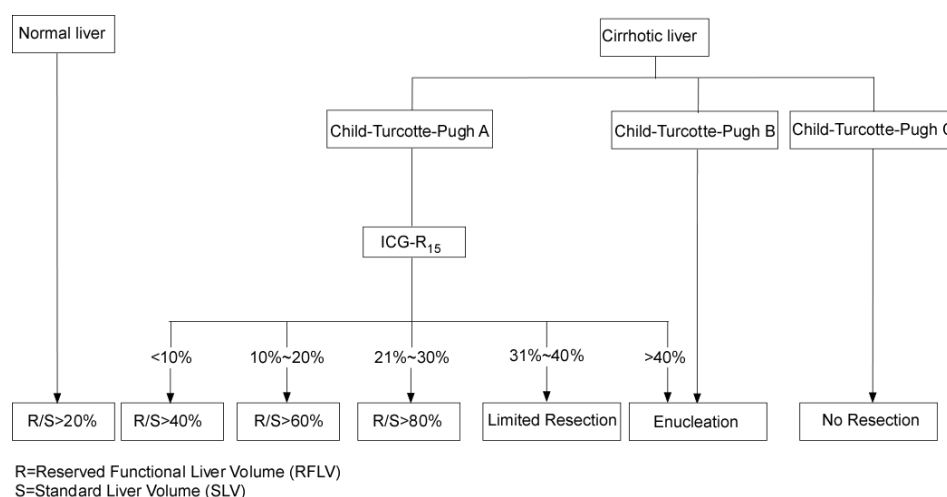
**Keywords:** Gastric cancer, incidence, mortality, gastrectomy, digestive tract reconstruction

## Safety validation of different decision trees in patients with liver tumore using fifty-fifty criteria

Xianqiang Kai, Jiangying Luo, Wenping Lv, Jiahong Dong\*

Department of Hepatobiliary Surgery, Chinese PLA General Hospital, Beijing China. E-mail: dongjh301@163.com.

**Background:** Although many criteria have been established for safe hepatic resection, evaluation of post-hepatectomy liver failure (PHLF) associated with several competing decision trees remains unknown. We have establish a decision tree for safe hepatectomy based on four variables: normal or cirrhotic liver, Child-Turcotte-Pugh score, the indocyanine green retention rate at 15 minutes (ICGR-15), and the ratio of reserved functional liver volume (RFLV) to standard liver volume (SLV).



**Methods:** A total of 2,457 patients underwent hepatic resection between January 2004 and December 2010 in Chinese PLA General Hospital, and 634 patients with live tumor were eligible for final analyses. PHLF has been identified by the association of prothrombin time < 50% and serum bilirubin > 50 micromol/L (the "50-50" criteria), which were assessed at day 5 postoperatively or later. Swiss-Clavien decision tree, Tokyo University-Makuuchi decision tree and Peking-Dong decision tree were adopted to divide patients into two groups on whether met those decision trees in sequence and PHLF rate were recorded.

**Results:** The overall mortality and PHLF rate were 4.1% and 3.0%.19 patients occurred PHLF, of which 8 patients died. Patients met Swiss-Clavien, Tokyo university-Makuuchi and Peking-Dong decision tree were 581, 573 and 622, whose PHLF rate were 2.75%, 2.62% and 2.73%, respectively. Cases satisfied Peking-Dong decision tree were significantly more than Swiss-Clavien decision tree and Tokyo university-Makuuchi decision tree ( $p = 0.000$ ,  $p = 0.000$ ), nevertheless the latter two shared no difference ( $p = 0.147$ ). The PHLF rate has no significant difference with respect to three decision trees.

**Conclusions:** Peking-Dong decision tree expands indications for hepatic resection for liver tumor, and not increases the PHLF rate comparing with Swiss-Clavien and Tokyo university-Makuuchi decision trees. It would be a safe and effective algorithm for hepatectomy in patients with liver tumor.

## **Liver transplantation for hepatocellular carcinoma: Japanese experience**

Sumihito Tamura<sup>1</sup>, Yasuhiko Sugawara<sup>1,2,4</sup>, Noriyo Yamashiki<sup>1,3,4</sup>, Kayo Nojiri<sup>4</sup>, Junichi Kaneko<sup>1</sup>, Taku Aoki<sup>2</sup>, Yoshihiro Sakamoto<sup>2</sup>, Kiyoshi Hasegawa<sup>2</sup>, Wei Tang<sup>2</sup>, Norihiro Kokudo<sup>1,2,4</sup>

1. *Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*
2. *Hepato, Biliary, Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*
3. *Department of Gastroenterology, The University of Tokyo Hospital, Tokyo, Japan.*
4. *Organ Transplantation Service, The University of Tokyo Hospital, Tokyo, Japan.*

Treatment of hepatocellular carcinoma (HCC) among patients with poor liver function continues to be a challenge. Liver transplantation is the most effective option when donor is available. From a global point of view, Japanese experience continues to be unique in two aspects.

First, the majority is based on living donors not restricted by nationwide deceased donor organ allocation system. It is now accepted in Japan that strict adherence to the Milan criteria (MC: one lesion smaller than 5 cm, up to 3 lesions smaller than 3 cm, no extra-hepatic manifestations and no vascular invasion) is no longer an absolute but a relative issue in the living donor setting. Second, HCV is more common rather than HBV compared to other Asian countries such as South Korea, or People's Republic of China. The inevitable recurrence of HCV under small graft size in the living donor setting remains problematic. Majority of HCV is genotype 1b with poor response to standard treatment. Various modified regimen of interferon treatment has been applied, including pre-emptive approach. Effect of these differences on the long term outcome awaits cautious analysis.

The advantage of living donor is that issue of organ allocation based on size-number criteria that limits organ allocation can be circumvented. Also, transplantation may be performed under a scheduled condition following detailed evaluation and optimized adjuvant treatment. A large survey consisted of 49 Japanese centers has been conducted, and a total of 653 patients with HCC that received living donor liver transplantation (LDLT) has been analyzed. HCV infection was a leading cause of liver cirrhosis, occurring in 385 recipients (59%) versus HBV for only 199 (30%). Actuarial patient survival was 72.6% at 3 years, and 68.9% at 5 years, and actuarial disease-free survival was 65.1% at 3 years, and 61.5% at 5 years. Most interestingly, 316 patients (48.4%) were beyond the Milan criteria. The recurrence rate of the patients who were beyond the Milan criteria were 17.7% at 1 year, 34.2% at 3 years, 37.3% at 5 years.

At The University of Tokyo, we have applied Living donor liver transplantation to HCC exceeding the MC in selected cases. The current guideline for HCC is up to 5 nodules with a maximum diameter of 5 cm (5-5 rule). To date, 120 patients with HCC underwent LDLT. Of these, 114 (95%) were within Tokyo 5-5 rule criteria and 104 (87%) were within MC. Seventy-six (63%) presented with HCV infection. Overall survival at 3 years and 5 years after transplantation were 81% and 78% (median follow up period 68 months). HCC recurred in 10 patients. Cumulative rates of recurrence at 3 years within and beyond Tokyo 5-5 rule or MC were 7% and 50%, or 8% and 20%.

Based on each individual center experience and registry data, it is now accepted in Japan that living donor transplantation continues to be the most successful option for HCC cases including those with HCV infection and other end-stage liver diseases. Further extension of size-number criteria and optimal treatment for co-existing HCV, however, still remains a matter of debate.

## **Evidence-based clinical practice guidelines: Standardized management for hepatocellular carcinoma**

Peipei Song, Wei Tang\*, Norihiro Kokudo

*Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths around the world, and more than 600,000 deaths are reported globally each year. Many studies worldwide have examined the management of HCC over the past 10 years. Following a management model, guidelines were defined as "systematically developed statements to assist practitioner and patient decisions about appropriate healthcare for specific clinical circumstances". With the development of evidence-based medicine (EBM), the concept of "transfer of current best evidence into clinical decision-making" has garnered substantial attention worldwide. As such a good tool, many clinical practice guidelines (CPGs) for HCC have been published worldwide under the guide of current best evidence. To evaluate the management of HCC worldwide, our study group did an English language literature search on the topic of guidelines or consensus for HCC published in the PubMed database during the period of 2001 to 2011. After a second screening, 46 articles were adopted from 3,008 hits to form 17 current guidelines for HCC around the world according to the selection criteria of credibility, influence, and being multi-faceted, including 5 guidelines from America, 7 from Asia, and 5 from Europe. We did a systematic evaluation on 17 current guidelines for HCC, which found that these guidelines have both similarities and differences in terms of what organizations or bodies drafted the guidelines and the approach, applicability, content, and recent updates of the guidelines as well as in terms of diagnostic and treatment algorithms. The differences could be attributed to various aetiological factors, high-risk patients, health systems, health resources, medical technology, treatment choices and income levels in different countries. Besides, although the full implementation of guidelines could benefit clinicians, patients and authorities, there is still a gap between projected goals and implementation. The comparative analysis of projected goals and implementation of guidelines for HCC showed that evidence-based CPGs for HCC are urgently needed and the appropriate constructing approach is the most important factor that influences guideline implementation. In order to achieve the current best evidence and promote evidence-based CPGs to be widely accepted and fully implemented, we recommend to conduct a systematic approach with 4 steps of global guidelines assessment, systematic literature review, experts' consensus and draft implementation, as well as implementation evaluation and periodic update in constructing and implementing evidence-based CPGs for HCC.

## **Transarterial chemoembolization (TACE) combined molecular targeted therapy of Hepatocellular carcinoma**

Wentao Li, Zheng Yuan, Weijun Peng

*Fudan University Shanghai Cancer Center, Shanghai, China.*

Hepatocellular carcinoma (HCC) is the sixth most common cancer and is the third leading cause of cancer-related deaths worldwide. The continued rise in HCC incidence and mortality emphasizes the need for novel therapeutic approaches. The clinical evaluation and management of HCC encompasses a multidisciplinary assessment combining comprehensive therapeutic approaches along with cancer surveillance implementation. Therapeutic options available to patients with HCC span a spectrum that includes less invasive locoregional therapies that utilize localized thermal ablation or intra-arterial infusion and embolization therapies, potentially curative operative resection or transplantation, and more novel molecular-based targeted therapies.

The challenging aspect of HCC management revolves around optimizing available therapy for each individual patient, an often difficult task that involves selecting appropriate candidates for appropriate therapies in an effort to maximize benefits and minimize harms in an era of limited resources. The advent of locoregional therapies has generated greater treatment options for patients with HCC. Transarterial chemoembolization (TACE) is considered a locoregional palliative treatment and is commonly used in HCC tumors that are not suitable for surgical resection or curative therapy. The underlying mechanism by which chemoembolization achieves its therapeutic potential hinges on principles of cytotoxicity and induced ischemia. The concept of TACE involves localized intra-arterial injection of chemotherapy agents followed by angiographically induced ischemia via selective embolization of the tumor's vascular supply. Although there has been greater experience with conventional forms of lipiodol based TACE, the recent advent of embolic, drug-eluting microspheres offers a promising alternative. The success of drug-eluting spheres has resulted in replacement of conventional TACE at many institutions with this new therapy. As is the case with surgical approaches, the success of TACE depends on appropriate patient selection. Subsequent subset analyses demonstrated that, although TACE was generally associated with improved outcomes when compared with conservative symptom-directed therapy among nonsurgical candidates, the improved survival was most significant among patients without evidence of decompensated cirrhosis, vascular invasion, or extrahepatic spread of tumor.

The field of systemic therapies for the treatment of HCC has been limited. Traditional cytotoxic chemotherapy regimens have failed to demonstrate significant improvements in HCC survival, and their extensive toxicity profile further contributed to their lack of efficacy. However, this may be about to change with the advent of a new therapy that targets tumor cells by halting tumor proliferation and angiogenesis, in addition to increasing the rate of tumor-programmed cell death. In recent years several pathways that control angiogenesis and cell proliferation were identified. Research in the area of angiogenesis inhibition in the management of cancer has contributed to a novel approach for the treatment of HCC. One of the earlier models of success in targeting angiogenesis as a treatment approach for HCC, sorafenib, a multitargeted tyrosine kinase inhibitor, was the first molecular targeted therapy to be approved by the US Food and Drug Administration for the treatment of unresectable HCC. The SHARP trial demonstrated a 2.8-month survival advantage among patients with advanced HCC treated with sorafenib compared with best supportive care (10.7 vs 7.9 months; hazard ratio, 0.69;  $P < .001$ ). A subsequent randomized, phase III trial that evaluated sorafenib among Asians with advanced HCC demonstrated results similar to the SHARP trial, with significant improvements in both survival and time to progression. Mounting evidence clearly supports the efficacy of sorafenib in treatment of advanced



---

HCC among a more diverse patient cohort and current guidelines by the American Association for the Study of Liver Diseases recommend sorafenib as first-line therapy among patients with unresectable HCC who are not appropriate candidates for percutaneous ablation or TACE, but maintain preserved liver function. However, the problems seem to be with the degree-associated toxicity. This may have led to discontinuation of treatment in a relatively moderate number of cases. Most serious events recorded were cardiac in nature.

Given the success of sorafenib, several additional anti-angiogenic agents currently under investigation demonstrate great promise. Brivanib is an anti-angiogenesis agent that inhibits both fibroblast growth factor and vascular endothelial growth factor pathways. The major theoretical advantage of this dual inhibitory mechanism of action is its potential to overcome development of compensatory signaling and subsequent loss of drug efficacy. Other agents such as sunitinib (Sutent) and bevacizumab (Avastin) have been less successful in the management of HCC owing to treatment-related toxicities and lack of survival efficacy. Many additional targeted therapies are currently under investigation. As newer agents continue to push the limits of improving morbidity and mortality among patients with HCC, the role of these novel agents in conjunction with operative resection, liver transplantation, ablation therapies, and/or concurrent oral systemic therapies that target different components of malignancy progression in an adjuvant or neoadjuvant fashion will likely offer the most potential and is under active investigation.

In conclusion, whereas surgical resection and transplantation conventionally form the cornerstone of curative approaches, the advancement of locoregional therapies holds great promise in cure. Careful patient selection is crucial prior to transarterial chemoembolization, as the procedure may be associated with an increased risk of liver failure. Sorafenib should be the first-line treatment in patients with advanced and inoperable HCC. The role of sorafenib in the management of early stage HCC remains to be determined. The application of combination of sorafenib with TACE to the management of advanced HCC may offer not only benefits in prolonged survival, but holds great promise in adding to the curative armamentarium, should be the first-line treatment in these patients.

## **Clinical research on Intracavitary treatment with Cinobufacini injection for malignant pleural or peritoneal effusions**

Chao An, Qin Zhou, Minghuan Zuo, Quanwang Li, Min Jiang, Tao Sun, Li Xiao, Zhanbing Qiao, Kaiwen Hu

*Department of Oncology, Dongfang Hospital, Beijing University of Chinese Medicine, Beijing, China.*

*\* Address correspondence to: Dr. Hu Kaiwen, M.D., Ph.D., Beijing University of Chinese Medicine, Dongfang Hospital Cancer Center, 1-No. 6Fangxingyuan, Fangzhuang, Fengtai District, Beijing 100078, P.R.C. E-mail: kaiwenh@163.com*

**Objectives:** This study was conducted to evaluate efficacy and safety of intracavitary treatment with Cinobufacini injection for malignant pleural or peritoneal effusions, explored the possible mechanism preliminarily.

**Methods:** After puncture drainage most of pleural or peritoneal effusions, 30 patients underwent slowly intracavitary injection of dilute Cinobufacini through the catheters, 3 times per week for two weeks.

**Results:** Efficacy was evaluated by clinical observations, Ultrasound exam, color RBC of effusions, tumour markers, Karnofsky score and Chinese Medial syndrome scale. CR 1 case (3.33%), PR 6 cases (20%), NC 7cases (23.33%), PD 16 cases (53.33%), the total efficacy rate was 46.67% (14/30). The concentration of RBC and Tumor Makers in malignant pleural or peritoneal effusions had significant decrease after treatment.

**Conclusions:** Intracavitary treatment with Cinobufacini injection is a good option for those patient with local syndrome differentiation of malignant pleural or peritoneal effusions is damp-heat-toxin.

**Keywords:** malignant effusions, Cinobufacini injection, Intracavitary treatment, differentiation based on local syndromes

---

## **The prediction and treatment strategy for hepatocellular carcinoma in high risk recurrence after hepatectomy**

Tianqiang Song

*Department of Hepatobiliary Surgery, Tianjin Medical University Cancer Hospital (TJMUCH), Tianjin, China.*

**Background:** Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. As a result of recent progress in treatment methods, the prognosis of HCC has been more favorable than before. However, the long-term prognosis after resection of HCC remains unsatisfactory as a result of a high incidence of recurrence. Prediction and prevention of recurrence are the most important strategies to improve the long-term survival results.

**Objectives:** To evaluate the current knowledge on the risk factors for recurrence, efficacy of adjuvant therapy in preventing recurrence, and the optimal management of recurrence after resection of HCC.

**Methods:** A review of relevant English articles was undertaken based on a Medline search from January 1980 to July 2011 and case summary from author's hospital

**Results:** Venous invasion, satellite nodules, tumor size, tumor number, liver cirrhosis, liver function and advanced pTNM stage, are the best-established risk factors for recurrence. Microvascular invasion (MVI) is an accepted independent predictor of poor survival after liver transplantation. Now more and more studies have suggested that the presence of MVI is an independent predictor of poor survival after resection of HCC. From January 2009 to January 2011, 296 HCC cases received resections in TJMUCH were analyzed and confirmed MVI is an independent predictor of poor survival. Preoperative magnetic imaging and macroscopic tumor type were preoperative predictors of MVI in HCC patients.

There is a lack of convincing evidence for the efficacy of neoadjuvant or adjuvant therapy in preventing recurrence. Some studies suggested that postoperative hepatic arterial chemotherapy, systemic chemotherapy, Iodine -131-lipiodol therapy, interferon therapy and target therapy (sorafenib) might improve disease-free survival, but results were conflicting. From the data of TJMUCH, Iodine -131-lipiodol therapy and target therapy might have better results for postoperative recurrence in high risk populations.

**Conclusions:** Several Factors are related with recurrence of postoperative HCC. But venous invasion especially MVI have to be paid more attention. Some novel preventive approaches suggest a potential role. But large-scaled randomized trials are needed to define the benefit of these treatment modalities for recurrence. Currently, the most realistic approach in prolonging survival after resection of HCC is early detection in high risk patients and aggressive management of recurrence.

## Lectures

### Session II Cancer prevention and drug discoveries

#### **Towards quantitative endogenous network theory on cancer genesis and progression: Beyond “cancer as diseases of genome”**

P. Ao<sup>1</sup>, D. Galas<sup>2</sup>, L. Hood<sup>2</sup>, X.M. Zhu<sup>3</sup>

1. *Shanghai Center for Systems Biomedicine and Department of Physics, Shanghai Jiao Tong University, Shanghai, China.*
2. *Institute for Systems Biology, Seattle, USA.*
3. *GenMath, Seattle, USA.*

There has been a tremendous progress in cancer research. However, it appears the current dominant cancer research framework of regarding cancer as diseases of genome leads impasse. Naturally questions have been asked that whether it is possible to develop alternative frameworks such that they can connect both to mutations and other genetic/genomic effects and to environmental factors. Furthermore, such framework can be made quantitative and with predictions experimentally testable. In this talk, I will present our positive answer to this calling. I will explain on our construction of endogenous network theory based on molecular-cellular agencies as dynamical variable. Such cancer theory explicitly demonstrates a profound connection to many fundamental concepts in physics, as such stochastic non-equilibrium processes, “energy” landscape, metastability, etc. It suggests that beneath cancer’s daunting complexity may lie a simplicity that gives grounds for hope. The rationales behind such theory, its predictions, and its initial experimental verifications will be presented.

#### *References*

1. Cancer as Robust Intrinsic State of Endogenous Molecular-Cellular Network Shaped by Evolution. P. Ao, D. Galas, L. Hood, X.-M. Zhu. *Medical Hypotheses* 2008; 70:678–684. <http://dx.doi.org/10.1016/j.mehy>
2. Towards Predictive Stochastic Dynamical Modeling of Cancer Genesis and Progression. P. Ao, D. Galas, L. Hood, L. Yin, X.M.Zhu. *Interdiscip Sci Comput Life Sci.* 2010; 2:140–144. DOI: 10.1007/s12539-010-0072-3.
3. Orders of Magnitude Change in Phenotype Rate Caused by Mutations. P. Ao. *Cellular Oncology.* 2007; 29: 67-69. [http://arxiv.org/PS\\_cache/arxiv/pdf/0704/0704.0429v1.pdf](http://arxiv.org/PS_cache/arxiv/pdf/0704/0704.0429v1.pdf).

## Study of Na<sup>+</sup>/K<sup>+</sup>-ATPase as a therapeutic target of product that contain cardiac glycosides

Litao Xu, Zhiqiang Meng

*Fudan University Shanghai Cancer Center, 270 Dongan Rd, Shanghai, China.*

Within the past years, there has been a substantial increase in the number of studies observing the anti-proliferative effects of cardiac glycoside compounds extracted from plants and even animals. The core structure of cardiac glycoside consists of a steroidal framework, which is considered the pharmaco-target responsible for the activity of these compounds.

The previous studies indicated that cardiac glycoside is the inhibitor of Na<sup>+</sup>/K<sup>+</sup>-ATPase that consists of two types of subunits, designated  $\alpha$  and  $\beta$ . The  $\alpha$ -subunit has binding sites for ATP, Na<sup>+</sup>, K<sup>+</sup>, and cardiac glycosides. The expression of  $\alpha$  isoforms is different in human tissues. Variation in the expression of these isoforms also occurs in human cancers, which determine the sensitivity of human cancer cells to cardiac glycosides.

Huachansu (HCS), a Chinese medicine that comes from dried toad venom from the skin glands of *Bufo gargarizans* or *B. melanostictus*, has been used in the treatment of various cancers in China. The extract primarily contains indole alkaloids and steroidal cardiac glycosides (bufadienolides). It has been accepted that the non-polar fraction is the mainly anti-tumor fractions and it functions via Na<sup>+</sup>/K<sup>+</sup>-ATPase in tumor cells. Phase I clinical study involving patients with advanced hepatocellular carcinoma, non-small cell lung cancer, or pancreatic cancer, jointly conducted by Fudan University Cancer Hospital and The University of Texas MD Anderson Cancer Center, showed that HCS was well tolerated and had encouraging antitumor efficacy. However, the anti-cancer mechanism of HCS needs to be identified.

Bufalin, a major bioactive component of the Chinese medicine Huachansu, has been reported to exhibit significant antitumor activity against various cancer cell lines. Our previous study demonstrated that bufalin inhibited the growth of the hepatocellular carcinoma (HCC) cells in a dose-dependent manner, which correlated with expression level of Na<sup>+</sup>/K<sup>+</sup>-ATPase  $\alpha_3$  in HCC cells. We also found that bufalin and Huachansu can improve the effects of other therapy in vitro and in vivo. Bufalin can improve the anti-proliferative activity of sorafenib in the human hepatocellular carcinoma (HCC) cell lines by contributing to the down regulation of ERK in vitro. And in vivo, sorafenib plus HCS synergistically inhibited the smmc7721 xenograft tumor growth. In another study, we also found that bufalin improve the hepatoma cells radiosensitivity in vitro by inhibiting the JAK2 /STAT3 signal transduction pathway and blocking the cells at the stages of G2/M.

Because chemotherapy has had limited benefits in most advanced malignancies, cardiac glycosides could be investigated for possible adjuvant therapy. Given that certain Na<sup>+</sup>/K<sup>+</sup>-ATPase subunits appear to make unique targets that are selectively expressed in tumor as opposed to normal tissue, cardiac glycosides should be considered as novel therapeutic agents for cancer therapy.

## **Design and prepare theranostic nano-vectors**

Na Zhang

*Department of Pharmaceutics, School of Pharmaceutical Science, Shandong University, Ji'nan, Shandong Province, China.*

**Objectives:** To design and prepare multifunctional nanoparticles as theranostic nano-vectors.

**Methods:** A novel biodegradable block polymer poly (lactic acid)-poly (ethylene glycol)-poly (l-lysine) (PLA-PEG-PLL) which contained free side chain amino groups were designed and synthesized. Diethylenetriaminepentaacetic acid (DTPA) and biotin were linked to the amino groups of PLA-PEG-PLL to form two modified materials: PLA-PEG-PLL-DTPA and PLA-PEG-PLL-biotin, respectively. The paclitaxel (PTX)-loaded nanoparticles (NPs) were prepared by solvent diffusion method with mixed PLA-PEG-PLL-DTPA and PLA-PEG-PLL-biotin where all the active groups (DTPA and biotin) were distributed on the surface of PTX-loaded NPs. Then Gd ions were chelated to the surface DTPA groups of the PTX-loaded NPs by incubating PTX-loaded NPs with GdCl<sub>3</sub> to obtain Gd, PTX-loaded NPs. The biotinylation alpha-fetoprotein (AFP) antibodies were linked to the surface biotin groups of Gd, PTX-loaded NPs by avidin-biotin reaction to obtain target Gd, PTX-loaded NPs.

**Results:** The target Gd, PTX-loaded NPs showed spherical morphology, positive surface charge, and uniform particle size distribution. The encapsulation efficiency and drug loading were  $88.76 \pm 1.64\%$  and  $1.59 \pm 0.06\%$ , respectively. The target Gd, PTX-loaded NPs showed high in vitro cytotoxicity and could be internalized by HepG2 cells and the internalization was both concentration and time depended. The target Gd, PTX-loaded NPs exhibited higher T1 relaxivity and higher cellular uptake rate compared to non-target Gd, PTX-loaded NPs. The in vivo MRI results indicated that the target Gd, PTX-loaded NPs showed significantly signal intensity enhancement at the tumor site compared with non-target Gd, PTX-loaded loaded NPs and Gd-DTPA injection (Magnevist<sup>®</sup>) in H22 tumor-bearing mice and the imaging time was significantly prolonged from less than an hour (Magnevist<sup>®</sup>) to 12 h. Meanwhile, the target Gd, PTX-loaded NPs showed significantly higher anti-tumor activity compared to PTX injection (Taxol<sup>®</sup>) and non-target Gd, PTX-loaded NPs.

**Conclusions:** These results demonstrated that the target Gd, PTX-loaded loaded NPs had great potential for theragnostic application on HCC.

**Keywords:** Theranostics, block polymers, paclitaxel, nanoparticles, MRI, hepatocellular carcinoma

## STAT3 as a target for HCC therapy

Xiaoxia Sun, Qiangjun Sui, Jian Zhang

*The institute of immunopharmacology and immunotherapy, Shandong University, Ji'nan, Shandong Province, China.*

STAT3 (signal transducer and activator of transcription 3) is an important transcriptional regulator of genes for control of cell growth, differentiation and apoptosis, including bcl-x1, cyclin D1, c-myc, mcl-1, VEGF, IL-10, TGF- $\beta$  and survivin. Constitutive activation of STAT3 has been found in a wide number of human primary tumors and cancer cell lines, and STAT3 is also noted to promote malignant transformation by modulating immune response. Inhibition of activated STAT3 would suppress the proliferation and induce apoptosis in many kinds of cancers. In our study, the STAT3 targeted “Decoy” oligonucleotides (STAT3 Decoy-ODN) was selected as the therapeutic to block STAT3 in HCC cells, and we found the proliferation of HCC cells was suppressed significantly by STAT3-decoy ODN *in vitro*, being associated with the increased apoptosis and cell arrest at G0/G1 to S phase transition. Further investigations showed the expression of STAT3-regulated genes including bcl-x1, cyclin D1 and c-myc, which involved in cell apoptosis and cell cycle progression, were down-regulated significantly both at transcription and translation levels. The anti-tumor effects were also confirmed in xenograft HCC nude mice. We found FITC labeled decoy ODN was efficiently transfected into cancer cells and induced apoptosis significantly. Compared with control, STAT3-decoy ODN could significantly inhibit the growth of tumor, the rate of inhibition was up to 87.5%, whereas there was no difference between scramble ODN and Lipofectamin treatment mice. Additionally, the weight of liver or spleen of decoy ODN treated mice decreased compare to other treatment mice, which was similar with no-bearing nude mice. Importantly, blocking STAT3 in HCC cells reversed the immunotolerance and improved the microenvironment by changing cytokine profile of HCC cells, downregulating immunosuppressive cytokines and upregulating immunostimulatory cytokines, and resulting in the augmentation of NK cell anti-tumor function. Finally, a safe, efficient, HCC targeted and pH sensitive gene delivery system was developed. These data suggested that STAT3 may be potentially used as a molecular target in HCC therapy.

## **Discovery of Anti-cancer Chemical Constituents from the Chinese Bryophytes**

Hongxiang Lou

*Department of Natural Product Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, Jinan 250012, P. R. China. E-mail: louhongxiang@sdu.edu.cn*

Bryophytes offer a rich source of rare and structurally unique molecules and can serve as a reservoir of potentially anti-cancer compounds for further development as pharmaceuticals. This report covered the chemical and biological research of Chinese bryophytes recently finished in our laboratory. A total of about 300 compounds, including more than 90 novel bisbenzyls and terpenoids, have been isolated and identified from 20 bryophyte species collected from North to South of China. The anti-proliferative effects of these isolated compounds against several cancer cell lines were evaluated. The anti-cancer modes of action of the bisbenzyls marchantin C and riccardin D were also investigated.



---

## Diterpenoids with Anti-cancer Potential from Chinese Medicinal Plants

Xiaoning Wang, Bo Wang, Hongxiang Lou

*Department of Natural Product Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44 West Wenhua Road, Jinan 250012, P. R. China. E-mail: wangxn@sdu.edu.cn.*

Ten new clerodane-type diterpenes, caseabalansins A-J, as well as seven known diterpenoids, were obtained from the twigs and leaves of *Casearia balansae*. Four new rearranged abietane diterpenoid hydroquinones, ajudecumins A-D, together with two known rearranged abietane diterpenoids, ajuforrestins A and B, three *neo*-clerodane diterpenoids, dihydroclerodin, clerodinins C and D, were isolated from the aerial parts of *Ajuga decumbens*. The structures of the new compounds were established on the basis of extensive MS and NMR spectroscopic data analysis and single-crystal X-ray diffraction analysis. Most of the clerodane-type diterpenoids from *Casearia balansae* showed significant cytotoxic activity against the four tumor cell lines PC3, DU145, SKOV3, and A549. Moreover, ajudecumins A and C from *Ajuga decumbens* exhibited inhibitory activity on the proliferation of human breast cancer MCF-7 cells.

## Oral presentation

### Session I Cancer treatment and cancer diagnosis

#### Real-time identification of pancreatic leak following pancreatic resection using fluorescent probes activated by chymotrypsin and $\gamma$ -glutamyltranspeptidase

Suguru Yamashita<sup>1</sup>, Takeaki Ishizawa<sup>1</sup>, Masayo Sakabe<sup>2</sup>, Junichi Kaneko<sup>1</sup>, Sumihito Tamura<sup>1</sup>, Yoshihiro Sakamoto<sup>1</sup>, Taku Aoki<sup>1</sup>, Yasuhiko Sugawara<sup>1</sup>, Kiyoshi Hasegawa<sup>1</sup>, Yasuteru Urano<sup>3,4</sup>, Norihiro Kokudo<sup>1</sup>

1. Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
2. Graduate School of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
3. Laboratory of Chemical Biology and Molecular Imaging, Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
4. Basic Research Program, Japan Science and Technology Agency, 7 Gobancho, Chiyoda-ku, Tokyo 102-0076, Japan.

**Background & Aims:** Pancreatic fistula (PF) remains one of the most serious complications after digestive surgeries, but is difficult to predict or prevent, mainly because there are no techniques to identify pancreatic juice leakage from the pancreatic stump or to rapidly evaluate the protease activities of leaking fluid that can cause symptomatic postoperative PF. The aim of this study was to develop novel fluorescent probes enabling real-time identification of pancreatic leak (PL) through rapid biochemical reactions with pancreatic enzymes.

**Methods:** The fluorescent intensities (FIs) of  $\gamma$ -glutamyl hydroxymethyl rhodamine green (gGlu-HMRG, activated with  $\gamma$ -glutamyltranspeptidase) and trypsin-added glutamyl-phenylalanine HMRG (gPhe-HMRG-Try, activated with chymotrypsin which is derived from chymotrypsinogen and trypsin) in pure pancreatic juice ( $n = 15$ ) and other intestinal or abdominal fluids drained after pancreatic resections ( $n = 61$ ) were evaluated to determine the fluid amylase levels. In the 30 consecutive patients, gPhe-HMRG-Try was sprayed on to a paper transcribing the pancreatic stump, and the ability to visualize PL from the stump and to predict postoperative PF were assessed.

**Results:** The FI of gPhe-HMRG-Try after 30 min incubation with the surgically drained fluids showed positive correlations with the fluid amylase levels ( $\rho = 0.678$ ,  $P < .001$ ); the FI of this probe was significantly higher than the FI of gGlu-HMRG in pure pancreatic juice (0.586 [0.144-1.23] arbitrary unit (a.u.) vs. 0.296 [0.033-0.594] a.u.,  $P = .011$ ). When gPhe-HMRG-Try was sprayed on to papers transcribing the pancreatic stump, its fluorescence in the pancreatic juice in the main pancreatic duct or its branches was identifiable by gross examination within a minute of the spraying in 23 of the 30 patients, but not in the remaining 7 patients; postoperatively, symptomatic PF developed only in the former group (incidence, 57% vs. 0%,  $P = .008$ ).

**Conclusions:** The gPhe-HMRG-Try probe has the potential to allow visualization of PL to be arrested during pancreatic/gastrointestinal tract surgery, and also allows rapid determination of the protease activities in the leaked pancreatic juice and drained abdominal fluids during such surgeries; thus, the use of this novel probe is expected to result in the possibility of reduction in the incidence of symptomatic PF and operative mortality after pancreatic surgery.

---

## Validation of a preoperative predictive scoring system for postoperative pancreatic fistula after pancreaticoduodenectomy

Ti Zhang, Huikai Li, Qiang Li

Department of Hepatobiliary Surgery, Tianjin Medical University Cancer Institute and Hospital, Key Laboratory of Cancer Prevention and Therapy, Tianjin, China, 300060. Corresponding author: LI Qiang, Email: liqiang4016@yahoo.com.

**Purpose:** To validate a preoperative predictive scoring system established by Japanese clinicians for postoperative pancreatic fistula (POPF) after pancreaticoduodenectomy (PD).

**Methods:** Data from 100 consecutive patients with periampullary tumor who underwent PD in Tianjin Medical University Cancer Institute and Hospital between October 2008 and January 2012 were reviewed. Factors potentially associated with POPF were analyzed by using chi-square Fisher exact test. The sensitivity and specificity of this system were determined by receiver operating characteristic (ROC) curve analysis.

**Results:** The incidence of POPF was higher in male than in female (17/62 Vs 3/38,  $P < 0.05$ ). The hard pancreas had the lowest POPF rate (0/16), while soft and medium hard pancreas accounted for the high POPF rate (14/47, 6/37), which had statistical significance ( $P < 0.05$ ). Patients with pancreatic cancer had lower POPF rate than non-pancreatic cancer (3/45 vs 17/55,  $P < 0.01$ ). Portal vein involvement was negative factor for the occurrence of POPF (6/67 vs 14/33,  $P < 0.01$ ). Anastomosis methods were also found to be associated with POPF. Duct to mucosa pancreaticojejunostomy (2/31) had lower POPF rate compared with End to End (11/50) or End to Side (7/19) anastomosis ( $P < 0.05$ ). ROC curve analysis showed that the sensitivity and specificity of this system were 95% and 96.3%, respectively. The nomogram showed an area under the curve (AUC) of 99% ( $P < 0.01$ ).

**Conclusions:** With satisfactory sensitivity and specificity, this scoring system can predict the occurrence of POPF and deserves wide clinical application.

**Keywords:** postoperative pancreatic fistula, prediction, pancreaticoduodenectomy

## Laparoscopic radio frequency ablation for hepatocellular carcinoma of specific location

Yuesi Zhong<sup>1,3\*</sup>, Meihai Deng<sup>1</sup>, Heping Fang<sup>1</sup>, Kai Li<sup>2</sup>, Ruiyun Xu<sup>1</sup>

1. Department of Hepatobiliary Surgery, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China.
2. Department of ultrasonography, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China.
3. Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

**\*Corresponding author:** Dr. Yuesi Zhong, Department of Hepatobiliary Surgery, Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, China, and Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan. E-mail: zhongyuesi@gmail.com

**Purpose:** To prospectively compare laparoscopic radio frequency ablation and percutaneous radio frequency ablation for hepatocellular carcinoma of specific location, such as tumor adjacent to the gallbladder, the stomach, the diaphragm, the flexura hepatica coli, or the right kidney.

**Materials and methods:** The ethics committee of the study institution approved the study protocol. Written informed consent was obtained from all patients at enrollment. 39 cases with tumors of specific location was included, all of the tumors of specific location were  $\leq 2$  cm adjacent to organs mentioned above. 25 cases received laparoscopic radio frequency ablation while 14 cases received percutaneous radio frequency ablation without laparoscopic assist. In the laparoscopic group, 8 of the 25 cases whose tumor adjacent to the gallbladder received laparoscopic cholecystectomy after the radio frequency ablation. 6 of the 14 cases of percutaneous group received absolute alcohol injection immediately after the ablation intraoperatively because of the inadequate ablation. 39 cases were all with liver cirrhosis, the number of tumors for each case was  $\leq 2$ , and the diameter of each tumor was  $\leq 3$  cm, All the cases of radio frequency ablation were ultrasound guided and performed with cool-tip needle technique. And the laparoscopic ultrasonic transducer was applied in laparoscopic group. The incidences of complications were observed, and the residual of tumors were observed 3 days after ablation with ultrasonic visualization.

**Results:** Laparoscopic and percutaneous group were with no significant difference of age and gender of cases ( $p > 0.05$ ). The location and number of tumors between these two groups were not significant different ( $p > 0.05$ ). There was no mortality in these two groups. In percutaneous group of 14 cases one case complicated with gastric perforation, one case complicated with perforation of gallbladder, and two cases complicated with perforation of diaphragm, these two cases of perforation of diaphragm presented with diaphragmatic hernia and biliary-bronchi fistula respectively. The incidence of complication of percutaneous group was 28.57%, however, in laparoscopic group, there was no similar complication ( $p < 0.002$ ). 3 days after ablation, the residual of tumors were observed. 4 of the 14 cases of percutaneous group revealed inadequate ablation and received absolute alcohol injection after ultrasonic visualization, while 2 of the 25 cases of laparoscopic group revealed inadequate ablation and received absolute alcohol injection. The incidence of residual of tumor of percutaneous group was higher than laparoscopic group ( $p = 0.02$ ).

**Conclusions :** Laparoscopic radio frequency ablation is safer and more effective than percutaneous radio frequency ablation without laparoscopic assist for hepatocellular carcinoma of specific location. It can help real-time monitoring and minimize the incidence of complications, so that adequate ablation can be

achieved to tumors. It is strongly recommended that laparoscopic ultrasonic transducer should be applied intraoperatively, which can minimize the affect of pneumoperitoneum.

**Acknowledgement:** *This work was supported by the National Natural Science Fund for Young Scholars of China (81000177, Yuesi Zhong) and supported in part by the Japan-China Medical Association.*

## ICG-fluorescent imaging during liver surgery for evaluation of portal uptake function in veno-occlusive regions

Yoshikuni Kawaguchi, Takeaki Ishizawa, Yoichi Miyata, Suguru Yamashita, Koichi Masuda, Shoichi Satou, Sumihito Tamura, Junichi Kaneko, Yoshihiro Sakamoto, Taku Aoki, Kiyoshi Hasegawa, Yasuhiko Sugawara, Norihiro Kokudo

*Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo, Japan.*

**Introduction:** Recent advances in imaging studies based on preoperative three-dimensional computed tomography (3D-CT) enables accurate estimation of liver volumes with veno-occlusive regions caused by resection of the major hepatic veins, which can affect postoperative liver function. However, it remains unclear to what extent hepatic function in such venous congested regions actually decrease compared with that in non-veno-occlusive regions. The aim of this study is to evaluate the regional differences in the portal uptake function in the remnant livers or transplanted liver grafts by visualizing the distribution of ICG to the entire liver surface following intravenous injection, using a commercially-available fluorescent imaging system.

**Methods:** In 63 patients who underwent liver resection with excision of the major hepatic veins ( $n = 22$ ) or liver transplantation (recipient,  $n = 23$ ; donor,  $n = 18$ ), ICG ( $2.5 \mu\text{g}$  per  $1\text{mL}$  of the remnant liver volume estimated with preoperative 3D-CT) was injected intravenously following resection, harvesting of the liver graft, or reconstruction of hepatic vessels in recipient. Then, fluorescent intensity (FI) on liver surfaces was recorded with fluorescent imaging system up to 300 seconds after the injection of ICG, and compared between veno-occlusive regions and non-veno-occlusive regions.

**Results:** The FI values on the liver surface increased linearly and then reached a plateau, providing a clear demarcation between the veno-occlusive regions and non-veno-occlusive regions. Plateau ICG concentrations converted from FI values were significantly lower in the veno-occlusive regions ( $C_{VO}$ ) than in the non-veno-occlusive regions ( $C_{Non}$ ) in liver resection patients (median [range],  $0.75 [0.29-2.0] \mu\text{g/mL}$  vs.  $3.0 [0.46-6.4] \mu\text{g/mL}$ ,  $P < 0.001$ ), donors ( $0.69 [0.29-1.9] \mu\text{g/mL}$  vs.  $2.4 [0.46-6.4] \mu\text{g/mL}$ ,  $P < 0.001$ ), and recipients ( $0.75 [0.34-1.8] \mu\text{g/mL}$  vs.  $1.8 [0.54-6.4] \mu\text{g/mL}$ ,  $P < 0.001$ ). Distributions of the  $C_{VO}/C_{Non}$  and the ratio of the hepatic uptake rate constant in the veno-occlusive regions to that in non-veno-occlusive regions were both around 40% (mean  $\pm$  standard deviation,  $0.36 \pm 0.17 \mu\text{g/mL}$  and  $0.42 \pm 0.16 \text{ sec}^{-1}$ , respectively). When the functional remnant liver volume was calculated as a sum of non-veno-occlusive regions and veno-occlusive regions multiplied by  $C_{VO}/C_{Non}$ , its ratio to the total liver volume was correlated with the improved postoperative/preoperative ratio of prothrombin time.

**Conclusions:** Portal uptake function in veno-occlusive regions is approximately 40% of that in non-veno-occlusive regions. Evaluation of portal uptake function using intraoperative ICG-fluorescent imaging enables accurate estimation of functional remnant liver volume, enhancing the safety of liver resection and transplantation.

---

## **Risk factors for the postoperative hemorrhage of digestive tract in patients with malignant obstructive jaundice**

Dalu Kong, Qiang Li, Xishan Hao

*Department of Hepatobiliary Surgery, Tianjin Medical University Cancer Hospital, Tianjin, China.*

**Objectives:** To investigate the correlative factors of postoperative hemorrhage of digestive tract in patients with malignant obstructive jaundice.

**Methods:** Between Jan 1985 and Jan 2005, 1345 patients with malignant obstructive jaundice were retrospectively analyzed regarding risk factors of the postoperative hemorrhage of digestive tract. Logistic regression was then used to determine the effect of multiple factors on postoperative hemorrhage of digestive tract.

**Results:** The hemorrhage of digestive tract appeared in 156 patients (11.6%). According to stepwise regression analysis, the risk factors for upper gastrointestinal bleeding after operation included suffering from accompanied ulcer diseases, radical operation, high TBL before operation.

**Conclusions:** The main reasons of hemorrhage of digestive tract after the operation for obstructive jaundice were the stress ulcer of digestive tract. Its incidence and mortality could be reduced by good preoperative preparations, sufficient oxygen supply during the operation, anti-infection treatment and inhibiting gastric acid secretion after operation.

## **Intraperitoneal injection of cinobufatacini and cancerous ascites treated by double-pathway chemotherapy**

Yinyin Li<sup>1</sup>, Yongping Wang<sup>1</sup>, Xiumin Yuan<sup>1</sup>, Qiang Liu<sup>1</sup>, Dongping Liu<sup>2</sup>

1. Chest hospital cancer treatment center in Shenyang, Shenyang, China;

2. China Medical University the first clinical College of Gastroenterology, Beijing, China.

**Purpose:** observation of intraperitoneal injection of cinobufatacini and cancerous ascites treated by double-pathway chemotherapy curative effect.

**Methods:** Control group: systemic chemotherapy at the same time fully after paracentesis,,Intraperitoneal perfusion of warm saline solution dissolved DDP 60mg, once a week, used after a two-week rest period of one week for one week. Observation group: two-pathway chemotherapy same as the control group, while cinobufatacini 100 mL intraperitoneal injection once a week, use two weeks, two weeks after a period of evaluation of curative effect.

**Results:** observation of effect of group compared with the control group, KPS rating, cytological changes of tumor markers of negative conversion rates and indexes are a statistically significant rise in  $p < 0.05$ .

**Conclusions:** Intraperitoneal injection of cinobufotalin combined with double-pathway chemotherapy is better methods of treatment of cancerous ascites.



---

## Hepatoblastoma in adult age: A report of 15 cases and review of literature

Qiang Wu

*Department of Hepatobiliary Surgery, Laboratory of Cancer Prevention and Therapy, Tianjin Medical University Cancer Institute and Hospital, Tianjin 300060, People's Republic of China.*

**Purposes:** Hepatoblastoma in adults is extremely rare and few cases have been reported in literature. We aimed to summarize the clinicopathological features and treatment for patients with hepatoblastoma in adults.

**Methods:** From 1963 to 2011, there were 15 patients with adult hepatoblastoma who underwent surgical treatment in Tianjin Cancer Hospital. Multiple demographic and clinicopathological parameters were reviewed retrospectively and compared with 23 cases of adult hepatoblastoma reported in English literatures since 1990.

**Results:** The mean ages of patients with adult hepatoblastoma were younger in the current study ( $50.1 \pm 12.5$  vs.  $39.2 \pm 11.7$ ), and the male:female ratios were also differed (8:7 vs. 10:13). The hepatitis virus infection was similar between groups (6:9 vs. 7:7). Pathologically, more patients in the current study had hepatoblastoma of epithelial type than in those of literature (epithelial type vs. mixed type: 11:4 vs. 7:11). Survival time was similar between two groups (30d-240m vs. 69d-151m). Surgical resection is the main effective treatment, but adjuvant therapy such as ablation and chemotherapy also benefit survival.

**Conclusions:** Adult hepatoblastoma has features of a short course, fast progress, high malignancy, and poor prognosis. Early diagnosis, a timely radical resection, close follow-up and adequate adjuvant chemotherapy are the key essentials that benefit survival.

**Key words:** Hepatoblastoma; Adult; Hepatectomy; Adjuvant Chemotherapy.

## Oral presentation

### Session II Drug Discoveries: therapeutic targets and pre-clinical study

#### Acetylated Hsp90 as a target for cancer therapy and drug development

Qiuyuan Peng, Bao Yong, Zhaohu Lin, Yonghua Yang

*School of Pharmacy, Fudan University, Shanghai, China.*

Heat shock protein 90 (hsp90) is a critical modulator in cell signaling processes to protect client proteins, such as oncoproteins and kinases, from misfolding and degradation. Over-expression of Hsp90 is common in both solid tumors and hematologic malignancies. Unlike targeting individual components of signal transduction pathways, Hsp90 inhibitors have the potential to target multiple oncogenic pathways simultaneously, by enhancing the ubiquitination and degradation of client proteins. Therefore, Hsp90 is an attractive target for cancer therapy and antitumor drug discovery.

So far, about 20 inhibitors targeting the N-terminal (ATP-binding domain) and carboxy-terminal of Hsp90 are now in development. However, these molecules targeting hsp90 have been the development of resistance caused by increased cellular levels of pro-survival chaperones. Also, a significant problem is that these inhibitors don't have the ability to discriminate normal cell and cancer cells.

Recently, we reported that Hsp90 function can be impaired by a post-translational modification, acetylation in vitro. Hyperacetylation of hsp90 was shown to inhibit the ATP, cochaperone p23, and client protein binding to hsp90, directing the client proteins to proteasomal degradation. Acetylation attenuates the binding of hsp90 with the client oncoproteins in cancer cells and site-specific acetylation stimulates the export of Hsp90 out of the cell and bound to the cell membrane to promote cancer cell metastasis.

In this study, Acetylated Hsp90 binding proteins have been identified inside cancerous cells from breast cancer patients. In particular, evidence will be provided for a true role of acetylated hsp90 in tumorigenesis in vivo and supporting that targeting acetylated Hsp90 may prove to be a therapeutically beneficial strategy for cancer therapy and an effective target for the development of antimetastatic agents.

## KL-6 mucin in hepatobiliary and pancreatic tumors

Yoshinori Inagaki, Wei Tang\*, Kiyoshi Hasegawa, Yasuhiko Sugawara, Norihiro Kokudo

*Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

KL-6 mucin, a kind of MUC1 mucin, has been clinically used as the diagnostic marker of interstitial pneumonitis. On the other hand, we previously showed that overexpression of KL-6 mucin was detected in various gastrointestinal cancers and related with worse survival of patients. However, the expression profile of KL-6 mucin in hepatobiliary and pancreatic cancer tissues and its clinical availability was not clear. In the present study, we performed the histochemical analysis of KL-6 mucin and evaluated relationship with clinicopathological characteristics. In primary liver cancer, expression of KL-6 mucin was detected in all cases of cholangiocarcinoma (CC) tissue but not in hepatocellular carcinoma (HCC) tissue. Furthermore, the level of KL-6 mucin in serum of CC patients was higher than that of healthy individuals or HCC patients, suggesting that KL-6 mucin is applicable to serological diagnosis for screening CC patients. In pancreatic tumor tissue, intraductal papillary mucinous tumor (IPMT) with high metastatic potential showed overexpression of KL-6 mucin. In addition, all cases of pancreatic ductal cancer tissues showed expression of KL-6 mucin. These results suggested that KL-6 mucin could be used for evaluation of worse behavior of pancreatic tumors. Then, we performed biological analyses using CC and pancreatic cancer cell lines. Treatment with *O*-glycan elongation inhibitor benzyl-alpha-*N*-acetylgalactosamine, which reduces the expression of KL-6 mucin, attenuated the invasive ability of CC cell line. In addition, down-regulation of MUC1 expression by siRNA decreased the invasive ability of pancreatic cancer cell line. Although further studies should be performed, the expression of MUC1 including KL-6 mucin may play an important role in cancer cell invasion. These results suggested that KL-6 mucin may be useful as the target of diagnosis and therapy for hepatobiliary and pancreatic tumors.

## **Identification of c-Met as a potential therapeutic target for hepatocellular carcinoma**

Jianjun Gao, Yoshinori Inagaki, Peipei Song, Wei Tang\*

*Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

The approval of sorafenib, an agent that targets receptor tyrosine kinases (RTKs), as the first effective drug for systemic treatment of HCC represents a milestone in treatment of this disease. As a typical member of the RTK family, c-Met represents an intriguing target for cancer therapy. However, the role of the c-Met signal transduction pathway is less unambiguous in HCC pathology, giving rise to concerns about the feasibility of utilizing c-Met targeting approaches for HCC treatment. Recently, basic studies on des- $\gamma$ -carboxy prothrombin, an abnormal cytokine secreted by HCC cells, by the current authors and other researchers have highlighted the critical role of c-Met signaling in HCC progression. In the current study, tissue expression features and serum levels of DCP were determined in HCC patients using immunohistochemical staining and enzyme-linked immunosorbent assay (ELISA), respectively. Results indicated that DCP was usually present in HCC tissues and in serum of HCC patients and was correlated with multiple clinicopathological characteristics. Moreover, expression of DCP in HCC tissues or presence of high serum levels of DCP indicated high malignancy of HCC. We next determined whether DCP and c-Met are co-expressed in HCC tissues using immunohistochemical staining. Results indicated that DCP and c-Met are commonly and concurrently expressed in HCC. Expression of neither DCP nor c-Met in HCC regions and adjacent regions signified a low rate of tumor recurrence after surgical resection. At last, we examined the anti-HCC effects of c-Met inhibitors and demonstrated that inhibiting c-Met kinase activities not only obviously suppressed the proliferation of HCC cells but also neutralized the growth stimulatory effects of DCP on HCC cells. The above results suggested that DCP and c-Met may constitute a autocrine signaling pathway, thus promoting HCC progression. Inhibiting c-Met kinase activities was a promising strategy for treatment of HCC.

---

## Design, synthesis and biological evaluation of 1, 2, 4, 7-substituted indole derivatives targeting serine/threonine kinase Akt/PKB

Peng Wang, Jianzhen Liu, Guisen Zhao\*

*Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, PR China. E-mail: guisenzhao@sdu.edu.cn*

Serine/threonine kinase Akt, also known as protein kinase B (PKB), is a central mediator of PI3K (phosphatidylinositol 3-kinase) and PTEN (phosphatase and tensin homolog deleted on chromosome 10) signaling pathway that plays an important role in multiple cellular functions such as cell growth, proliferation, migration, apoptosis and survival. Overexpression or overactivity of Akt is found in a variety of human cancers including breast, ovarian, prostate, pancreatic, and skin cancers. Therefore, inhibition of Akt is a strategy for molecular targeting therapy of cancer.

In order to discover novel Akt inhibitors, we designed and synthesized a series of 1, 2, 4, 7-substituted indole derivatives with the understanding of the reported co-crystal structures of Akt and its inhibitors, which occupied the ATP-binding site of Akt, acting as ATP-competitive inhibitors. The inhibitory effects of these compounds on Akt activity and on PC-3 cell proliferation were determined. The experimental data showed that some compounds (34b, 38ac, 38bb and 3bc) in this series exhibited more potent anti-proliferative ability in PC-3 cell line and more potent inhibition on the Akt1 activity *in vitro* than GSK690693.

**Acknowledgements:** This work was supported by the National Natural Science Foundation of China (No. 21072115) and Shandong Natural Science Foundation (No. ZR2011HM042).

## Usefulness of silkworm disease model for drug development

Kazuhisa Sekimizu

*Lab. of Microbiology, Grad. Sch. Pharm. Sci., Univ. of Tokyo, Tokyo, Japan.*

Preclinical tests using animal models are necessary for evaluating the therapeutic effects of drug candidates, since most drug candidates obtained by *in vitro* screening are inappropriate as medicines due to toxicity and their problems of pharmacokinetics in the human body. Although mammalian models, such as mice, rats, dogs, cats, and rabbits, have been used to examine toxicity and pharmacokinetics of drug candidates, both the high cost and the ethical issues of sacrificing mammals for drug analysis delay the development of potentially therapeutically effective drugs. The use of invertebrate animals for the evaluation of drug candidates can overcome these problems. We propose the use of silkworms, *Bombyx mori*, as model animals to evaluate the properties of drug candidates. The lower cost and smaller space required for the maintenance of silkworms compared to mice allows us for a larger number of animals to be handled in limited facilities. Because of the long history of the silk industry, the methods for taking care of silkworms are well established in Asian countries. Silkworms are ideal for use in a large-scale drug screening system, as they have large sizes enough to be used in injection experiments, for making hemolymph preparations, and for isolating organs such as the midgut, which are essential processes for studying the pharmacokinetics of drugs in individual animals. The silkworm has a number of cytochrome P450s and conjugation enzymes, which are involved in drug detoxification.

In this symposium, I report that establishment of the disease models such as infection model with pathogenic bacteria for humans, and hyperglycemic model. Furthermore, transgenic technology is applicable for silkworm. I will discuss our recent discovery of novel antibiotics 'Kaikosin' by silkworm *Staphylococcus aureus* infection model.

## Identification of novel hepatitis C virus NS3 inhibitors: Psammaplin A and Halisulfate 3

Kazi Abdus Salam<sup>1</sup>, Atsushi Furuta<sup>2,3</sup>, Naohiro Noda<sup>3,2</sup>, Satoshi Tsuneda<sup>2</sup>, Yuji Sekiguchi<sup>3</sup>, Atsuya Yamashita<sup>4</sup>, Kohji Moriishi<sup>4</sup>, Masamichi Nakakoshi<sup>5</sup>, Masayoshi Tsubuki<sup>5</sup>, Hidenori Tani<sup>6</sup>, Junichi Tanaka<sup>7</sup>, Nobuyoshi Akimitsu<sup>1</sup>

1. Radioisotope Center, The University of Tokyo, 2-11-16 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan;
2. Department of Life Science and Medical Bioscience, Waseda University, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8480, Japan;
3. Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan;
4. Department of Microbiology, Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Chuo-shi, Yamanashi 409-3898, Japan;
5. Institute of Medical Chemistry, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142-8501, Japan;
6. Research Institute for Environmental Management Technology, AIST, 16-1, Onogawa, Tsukuba, Ibaraki 305-8569, Japan;
7. Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan.

Hepatitis C virus (HCV) is one of the most causative agents of hepatitis C, a chronic infectious disease that can lead to development of hepatocellular carcinoma. The HCV viral genome is 9.6 kb that encodes at least ten viral proteins. Among them, the nonstructural protein 3 (NS3) of HCV consists of two domains. The N-terminal one third of NS3 protein has a serine protease activity, whereas C-terminal two-thirds have nucleoside triphosphatase (NTPase) and RNA helicase activity. Like other helicases, HCV RNA helicase possesses NTPase which is essential for their translocation and unwinding of dsRNA in a direction of 3' to 5'. Since both NTPase and RNA helicases are essential for viral replication, therefore, HCV NS3 NTPase/helicase is an attractive target for the development of antiviral therapy and can be considered to design NS3 inhibitors. In this study, we identified that psammaplin A and halisulfate 3 from marine sponge extracts as NS3 RNA helicase inhibitors using a high-throughput screening photoinduced electron transfer (PET) system that we previously developed.

We determined the inhibitory fashion of two inhibitors for the inhibition of NS3 ATPase by TLC analysis using radio-labeled ATP. Our results suggested that both psammaplin A and halisulfate 3 inhibited ATPase activity of NS3 in a dose dependent manner, with IC<sub>50</sub> of 32 and 8 μM, respectively. Next, we examined the inhibitory effects of psammaplin A and halisulfate 3 on NS3 RNA helicase activity using a dsRNA substrate with IC<sub>50</sub> 17 and 5 μM, respectively. We also evaluated whether they affect the binding of NS3 to RNA. Gel mobility shift assay was shown that psammaplin A and halisulfate 3 prevented the binding of NS3 to ssRNA, indicating that these two inhibitors might compete with RNA for the nucleic acid binding site on NS3. Furthermore, both inhibitors have been shown inhibitory effects on viral replication in subgenomic replicon cells.

## **Synthesis of dihydroxypyrimidine-4-carboxamide derivatives as HIV-1 integrase inhibitors**

Shenghui Yu<sup>1</sup>, Nouri Neamati<sup>2</sup>, Guisen Zhao<sup>1,\*</sup>

1. Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, PR China; E-mail: [guisenzhao@sdu.edu.cn](mailto:guisenzhao@sdu.edu.cn).

2. Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA.

Human immunodeficiency virus type -1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS). HIV-1 integrase is an essential enzyme that catalyzes the insertion of the viral NDA into the genome of the host cell through a complex process, which consists of three biochemical steps: (i) cleavage of a dinucleotide pair from the 3'-end of the viral DNA (termed 3'-processing), (ii) insertion of the resulting shortened strands into the host cell chromosome (termed strand transfer), and (iii) removal of the two unpaired nucleotides at the 5'-end of the viral DNA and gap-filling process. Due to the absence of any known human homolog, HIV-1 integrase is considered as an attractive and validated target for the development of novel anti-HIV drugs. The emergence of Raltegravir that is a derivative of dihydroxypyrimidine makes HIV integrase inhibitors gain a definitive place in the treatment of HIV infection. This encouraged us to design and synthesize a set of novel dihydroxypyrimidine-4-carboxamide derivatives. The ability of all the synthesized compounds to inhibit 3'-processing and strand transfer was tested. The experimental data showed that most compounds exhibited strand transfer inhibitory potency with IC<sub>50</sub> value less than 10 μM. Compounds 6-a5 and 6-a8 had the most potent inhibitory efficacy on strand transfer with an IC<sub>50</sub> value of 0.5 μM.

**Acknowledgements:** This work was supported by the National Natural Science Foundation of China (Grant No. 20872082 and 21072115).



## The multifunctional nanoscaled delivery system for codelivery of nucleic acids and chemotherapeutics

Chunxi Liu, [Na Zhang](#)

Department of Pharmaceutics, School of Pharmaceutical Science, Shandong University, 44 West culture Road, Ji'nan, Shandong Province, China.

Codelivery of different therapeutics has a potential to efficaciously treat human diseases via their synergistic effects. Activable therapeutic tools at the nanoscale are suitable platforms for codelivery of different therapeutics, including nucleic acids and chemotherapeutics. We have recently developed a multifunctional nanoscaled delivery system simultaneously applied with tumor-targeted, pH-triggered and codelivery strategies. In this study, DOX-HZN-PEI-PEG-TAT was first synthesized and complexed with pDNA to form DOX loaded-TAT modified polyion complex micelles (PIC). Next, sulfamerazine (SA)-PEG-NGR (SA, pKa = 7.0) was synthesized and attracted on the surface of PIC to obtain NGR modified PIC micelles (targeted PIC micelles, TPIC). SA is weakly acidic due to the readily ionizable hydrogen atom in the amide bond in water. Above the pKa, SA has a negative charge, while it is neutral below the pKa. Therefore, at physiological pH (pH = 7.4), the TPIC could be constructed by the electrostatic interaction between the positive charged core of PIC and negatively SA. Meanwhile, the steric hindrance created by the long PEG spacer was expected to shield the surface-attached TAT to reduce its associated side effects on normal tissue. While, at tumour site (pH < 7.0), SA-PEG-NGR could be dissociated from the surface of PIC and TAT was exposed to facilitate the tumour cellular uptake. The resultant DOX-HZN-PEI-PEG-TAT conjugates had a drug loading of  $8.01 \pm 1.22\%$ . The results of in vitro DOX release confirmed that the DOX release via pH-dependent hydrolysis of the hydrazone bond, which showed lower release rate at pH 7.4 and higher rate pH 5.0. In addition, DOX-HZN-PEI-PEG-TAT exhibited higher cell cytotoxicity at pH 5.0 than pH 7.4 ( $p < 0.05$ ) and higher fluorescence intensity in cells at pH 5.0 was shown than that at pH 7.4. The obtained PIC and TPIC displayed nearly spherical shape and no aggregation was observed. The sizes were  $187.6 \pm 4.3\text{nm}$  and  $221.2 \pm 6.8\text{nm}$ , respectively. The zeta potentials were  $20.5 \pm 2.4\text{mV}$  and  $-10.9 \pm 1.3\text{mV}$ , respectively. The results of in vitro cell studies indicated that both PIC and TPIC could successfully transfect HUVEC, MCF-7 and SKOV-3 cells. Compared with PIC, TPIC exhibited higher transfection efficiency in CD13<sup>+</sup> cells than CD13<sup>-</sup> cells. More importantly, both PIC and TPIC were able to simultaneously accomplish intracellular drug delivery and gene transfection, indicating that the co-delivery is a cooperative process. All in all, the novel multifunctional nanoscaled delivery system is promising carrier for the codelivery of nucleic acids and chemotherapeutic agents.

**Keywords:** Codelivery; multifunctional nanoscaled delivery system; doxorubicin; gene delivery; polyion complex micelles

## 13F-1, a peptidomimetics compound inhibited Colo205 cancer growth by targeting APN (CD13)

Hou Li Zhang, Shuxiang Cui\*

*Key Laboratory for Rare Disease of Shandong Province, Department of Pharmacology, Institute of Materia Medica, Shandong Academy of Medical Sciences, Jinan 250062, China. E-mail: sxccui@sina.com*

**Objectives:** 13F-1 is a novel peptidomimetics compound designed based on the structure of APN (CD13). 13F-1 has been considered to possess the anticancer activity by targeting APN (CD13). We aimed to examine the effect of 13F-1 by in vitro and in vivo studies.

**Methods:** In vitro studies, the human colonic adenocarcinoma cells (Colo205) were employed and the proliferation ability was identified by MTT assay. The fluorescent probe, DCFH-DA, was used to measure the level of ROS in cancer cells. The level of SOD was examined by the kit of SOD, using the method of WST-1. The fluorescent, lipophilic and cationic probe, JC-1, was used to detect the mitochondrial membrane potential ( $\Delta\Psi_m$ ). In vivo assays, nude mice bearing Colo205 xenografts were injected with 13F-1. Mice were sacrificed and cancer tissues were removed for weighing. The levels of SOD, ROS in Colo205 xenografts were examined using the kits of ROS and SOD. ELISA assay was used to measure the level of 8-OHdG in Colo205 xenografts. Immunohistochemical staining and western blot were used to determine the expressions of APN (CD13), Bax and Bcl-2.

**Results:** 13F-1 effectively inhibited the proliferation of Colo205 cells. The molecular analysis showed that the levels of ROS were significantly elevated, while the NAC was blocked ROS. Compared with negative control, SOD and  $\Delta\Psi_m$  were declined in 13F-1-treated cells. The effects of 13F-1 were confirmed in nude mice bearing Colo205 xenografts. 13F-1 at 50,100 and 150mg/kg inhibited cancer growth by 18.87%, 50.17% and 63.94% ( $P < 0.05$ ), respectively. Further examination indicated the increase of ROS and 8-OHdG in 13F-1-treated cancer tissues. Immunohistochemical staining indicated that 13F-1 reduced the levels of APN in the membrane of cancer cells. 13F-1 possessed the ability of Bax/Bcl-2 ratio, showing the increase of Bax and decrease of Bcl-2.

**Conclusions:** Our results suggested that 13F-1 possessed anticancer activity against human colon cancers. This effect might be due to the multiple mechanisms like induction of ROS, which caused cells apoptosis and oxidative DNA damage.

## Identifying and optimizing novel B-Raf<sup>V600E</sup> inhibitors by virtual screening

Zhijian Xu, Guirui Yan, Heyao Wang, Weiliang Zhu

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China (E-mail: wlzhu@mail.shenc.ac.cn).

The Ras/Raf/MEK/ERK pathway exists in all eukaryotes which pertains to growth control in all its facets. B-Raf, one of the three types of Raf proteins, was found in 66% of malignant melanomas with a single substitution (B-Raf<sup>V600E</sup>) accounting for 80%. Its *in vitro* kinase activity is ~500 fold greater than that of wild type B-Raf, which stimulates cancer cell proliferation and protects them from apoptosis. Therefore, B-Raf<sup>V600E</sup> is an attractive anticancer target for personalized therapy. For searching novel B-Raf<sup>V600E</sup> inhibitors, we constructed a pharmacophore model, a virtual screening was then performed based on the model, identifying a compound, **1**, as a hit with an IC<sub>50</sub> of 4 μM. However, the chemical structure of **1** is very complicated without novelty. To optimize its structure, we performed another virtual screening by keeping the essential hydrogen bonds formed by **1** with the hinge region of B-Raf<sup>V600E</sup>, leading to the discovery of some 2-thioxodihydropyrimidine-4,6(1*H*,5*H*)-dione derivatives. Bioassay results showed strong inhibitory activities of the derivatives. Using 5-(furan-2-ylmethylene)-2-thioxodihydropyrimidine-4,6 (1*H*,5*H*)-dione as a new scaffold, a comprehensive SAR analysis with 20 compounds were performed using substructure search. A compound was identified in this process, which is 10-fold more potent than the hit **1**, showing an activity comparable to that of vemurafenib. Therefore, this study demonstrated that it is an efficient way to identify novel potential B-Raf<sup>V600E</sup> inhibitors by combining pharmacophore, molecular docking, and substructure search approaches.

### References:

Zhijian Xu, Guirui Yan, Gaihong Wang, Bo Li, Jianming Zhu, Peng Sun, Xiaodong Zhang, Cheng Luo, Heyao Wang, , Weiliang Zhu, Combining pharmacophore, docking and substructure search approaches to identify and optimize novel B-RafV600E inhibitors, BMCL, 2012, in press.

## Study on the penetration and inhibition of angiogenesis of endostatin with the mediation of Tat PTD

Xinke Zhang<sup>1</sup>, Yan Li<sup>1</sup>, Haining Tan<sup>2</sup>, Yanna Cheng<sup>2,3</sup>, Fengshan Wang<sup>1,2,\*</sup>

1. Key Laboratory of Chemical Biology of Natural Products (Ministry of Education), Institute of Biochemical and Biotechnological Drugs, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, Shandong, China;
2. National Glycoengineering Research Center, Shandong University, Jinan 250012, Shandong, China;
3. Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, Shandong, China.

Endostatin(Es), the C-terminal fragment of collagen XVIII, is a potent inhibitor of angiogenesis and tumor growth. Poor permeability to cell membrane affects its efficacy and it has to be administered high doses to play its effects. In order to increase the cell membrane permeability of Es, we fused the protein with protein transduction domain of Tat (Tat PTD) in *Escherichia coli* BL21 (DE3) cells through genetic engineering. Most of the fusion protein (Tat PTD-Es) was expressed as inclusion bodies in *Escherichia coli* BL21 (DE3) cells, so Tat PTD-Es was prepared by a series of operation including inclusion body denaturation, refolding and chromatography. Tat PTD-Es showed more than 80% inhibition on EAHY926 cells proliferation at concentrations higher than 4 $\mu$ mol/L. The inhibition activity of Tat PTD-Es was observed on the angiogenesis of chorioallantoic membrane (CAM) induced by bFGF. Compared with bFGF group (30.74  $\pm$  6.58), the numbers of blood vessels of CAM treated with Tat PTD-Es and Es were reduced from to (12.18  $\pm$  4.92) and (14.56  $\pm$  6.21), respectively ( $p < 0.05$ ). The results showed that Tat PTD-Es could significantly inhibit angiogenesis of CAM induced by bFGF. In addition, the abilities of cellular internalization of Tat PTD-Es and Es were compared. The cellular translocating ability of Tat PTD-Es was much greater than that of Es. The percentage of positive cells after exposure to Tat PTD-Es and Es were 99.28% and 33.61%, respectively. Moreover, the uptake mechanism of this Tat PTD-mediated protein was extensively studied. The results revealed that different mechanisms were involved in the entry of Tat PTD-Es into cells. In *eye ball penetrating* studies, Tat PTD-Es appeared in retinal after eye-dropping which proved that Tat PTD could carry protein to penetrate eye barriers through eye-dropping. In the experiment using the mouse choroidal neovascularization (CNV) model showed that the area of CNV treated with Tat PTD-Es eye-dropping was (1378.4  $\pm$  154.3)  $\mu$ m<sup>2</sup>, which significantly decreased compared to negative control, of which the CNV area was (2623.6  $\pm$  240.9) $\mu$ m<sup>2</sup> ( $p < 0.01$ ). Tat PTD-Es via eye drops was able to inhibit CNV angiogenesis, and these results further demonstrated that Tat PTD-Es had the capacity to penetrate the eye barrier, reach the retina choroid and play its role via eye-dropping. Tat PTD-Es is also expected to penetrate tumor even blood-brain barrier (BBB) and could be more efficient in treating angiogenesis related diseases.

## Novel and potent HIV-1 NNRTIs research: Design, synthesis and biological evaluation

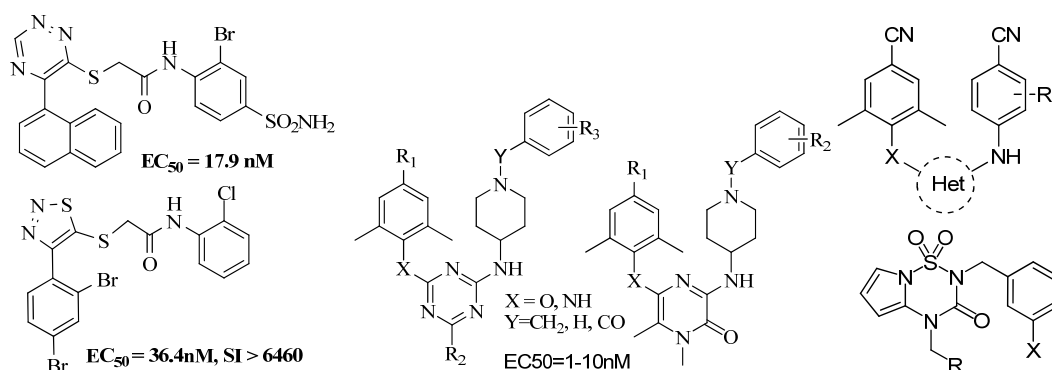
Xinyong Liu<sup>1,\*</sup>, Peng Zhan<sup>1</sup>, Xuwang Chen<sup>1</sup>, Wenmin Chen<sup>1</sup>, Ye Tian<sup>1</sup>, Xiao Li<sup>1</sup>, Christophe Pannecouque<sup>2</sup>, Jan Balzarini<sup>2</sup>, Erik De Clercq<sup>2</sup>

1. Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University (E-mail: xinyongl@sdu.edu.cn);
2. Rega Institute for Medical Research, K.U. Leuven, Belgium.

Acquired Immune Deficiency Syndrome (AIDS) caused by human immunodeficiency virus (HIV), is still prevalent worldwide. The most efficient and standard treatment regimen for HIV-1 infection, namely as highly active antiretroviral therapy (HAART), commonly involves two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitors (PIs) or a non-nucleoside reverse transcriptase inhibitors (NNRTIs). As a key component of HAART, NNRTIs play important role in the currently clinical AIDS therapies because of their high potency and specificity. However, the efficacy of NNRTIs is seriously hampered by the emergence of mutant viral strains. Therefore, there is a urgent need for next-generation NNRTIs with better resistance profiles and improved safety and tolerability.

As a result of coordinated multidisciplinary efforts, great achievements have been made in the discovery of new generation of NNRTIs. Currently, 5 NNRTIs have been approved by US FDA and many candidates have been undergoing clinical trials. Prompted by these promising results, and in continuation of our ongoing programs directed toward the development of novel anti-HIV agents, recently our group is engaged in several kind of structurally diversified NNRTIs, which were designed based on medicinal chemistry strategies, such as bioisosterism, molecular hybridization, scaffold hopping and fragment-based drug discovery, and structural biology (crystallography), as well as computational chemistry (molecular modeling). From biological assays, we had discovered some new and promising lead compounds to be potent and selective HIV-1 NNRTIs

(Figure.1)



### Reference:

1. Li D, Zhan P, Clercq ED, Liu X\*. J. Med. Chem., 2012; 55:3595.
2. Zhan, P.; Liu, X. Eur J Med Chem. 2011; 46:5039-5045.
3. Chen, X. W., Zhan, P., Liu, X. Y., et al., Eur. J. Med. Chem. 2012; 51:60.
4. Chen, X. W., Zhan, P., Liu, X. Y., et al., Bioorg. Med. Chem. 2012; 20:3856.
5. Zhan. P.; Liu, X.; Fang, Z.; et al., Bioorg. Med. Chem. 2009; 17: 6374.

## **Recombinant HDL nanoparticles for drug delivery and imaging of the liver**

Yuhong Xu, Mengjie Rui

*Shanghai Jiaotong University, Shanghai, China.*

Lipoproteins are a family of spherical macromolecular particles with the common structure of a hydrophobic core surrounded by a lipid monolayer embedded with apolipoproteins and cholesterols. They are characterized into several classes based on their composition and mass density. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are the most extensively studied lipoproteins. Significant efforts have been devoted to the use of recombinant lipoprotein-like nanoparticles as drug delivery vehicles and diagnostic agents, because most of these particles resemble natural lipoprotein structures and are considered highly biocompatible and safe.

We took advantage of such a structure and prepared two different gadolinium (Gd)-DTPA-labeled cholesterol-containing recombinant HDL nanoparticles (Gd-chol-HDL) and Gd-(chol) 2-HDL as liver-specific magnetic resonance imaging (MRI) contrast agents. The reconstituted HDL nanoparticles had structural similarity to native HDL, and could be taken up by HepG2 cells via interaction with HDL receptors *in vitro*. *In vivo* MRI studies in rats after intravenous injections of 10  $\mu\text{mol}$  gadolinium per kg of recombinant HDL nanoparticles indicated that both nanoparticles could provide signal enhancement in the liver and related organs. However, different T<sub>2</sub>-weighted image details suggested that they participated in different cholesterol metabolism and excretion pathways in the liver.

In addition, we have also developed a recombinant high density lipoprotein (rHDL) vector with high siRNA encapsulation efficiency. They were prepared by condensing siRNA with various commercial cationic polymers and coating the polyplex with a monolayer of lipids and apolipoprotein AI (apo AI). Characterization studies revealed these rHDL nanoparticles had similar physical properties as natural HDLs. The various rHDL formulations had high silencing efficiency (more than 70% knockdown) in hepatocytes with minimum cytotoxicity. Moreover, the uptake of rHDL by SMMC-7721 was confirmed to be mediated through the natural HDL uptake pathway. Therefore, the optimized rHDL nanoparticles may offer a promising tool for siRNA delivery to the liver.

## Effects of monoclonal antibodies, MLS128 against Tn-antigen and 1H7 against insulin-like growth factor-I receptor, on colon cancer cell growth

Normaiza Zamri<sup>1</sup>, Naoya Masuda<sup>1</sup>, Fumie Oura<sup>1</sup>, Yukiko Yajima<sup>1</sup>, Hiroshi Nakada<sup>2</sup>, Yoko Fujita-Yamaguchi<sup>1</sup>

1. Department of Applied Biochemistry, Tokai University School of Engineering, Kanagawa, Japan;

2. Department of Molecular Bioscience, Faculty of Life Sciences, Kyoto Sangyo University, Kyoto, Japan.

MLS128 is a Tn-antigen specific monoclonal antibody (mAb)<sup>1</sup>, which binds carbohydrate epitopes consisting of three or two consecutive Tn-antigens (Tn3 or Tn2)<sup>2-4</sup>. We previously showed that MLS128 bound 110-210 kDa glycoproteins and exerted growth inhibitory effects on LS180 and HT29 colon cancer cells as well as MCF-7 breast cancer cells<sup>5</sup>. Since insulin-like growth factor-I receptor (IGF-IR) down-regulation in LS180 cells was suggested as one possible mechanism for the observed growth inhibitory effects of MLS128<sup>5</sup>, the role of IGF-IR signaling in colon cancer cell growth and its possible interaction with MLS128-induced cancer cell growth inhibition were studied. Human colon adenocarcinoma LS180, LS174T, and HT-29 cells were used to determine effects of MLS128 or 1H7 (anti-IGF-IR mAb) on cell growth and mechanisms underlying their effects on the observed cancer cell growth. Results of this study demonstrated: 1) MLS128 or 1H7 treatment significantly inhibited all three colon cancer cell growth. 2) Three colon cancer cell lines expressed IGF-IR and the growth of three colon cancer cell lines was in part IGF-I dependent. 3) MLS128 didn't affect the downstream signaling stimulated by IGF-I as determined by phosphorylation of Tyr, MAPK, and Akt, suggesting that inhibitory effects of MLS128 on colon cancer cell growth is independent of IGF-IR signaling. 4) Three colon cancer cell lines expressed 110kDa glycoprotein (GP) as the binding site for MLS128. In contrast, breast cancer MCF-7 cells did not express the 110kDa GP but expressed MLS128-detectable bands with higher molecular masses. 5) Treatment of cells with 1H7 caused down-regulation of the IGF-IR without affecting 110kDa GP levels. 6) Treatment of cells with MLS128 resulted in partial disappearance of the 110kDa band without affecting IGF-IR levels. 7) Western blotting analyses of the colon and breast cancer cell lysates, to determine whether various levels of growth-related molecules are expressed in these cancer cells, clearly demonstrated (i) colon and breast cancer cells express significantly different sets of growth-related molecules, and (ii) although somewhat alike, expression patterns of those molecules among three colon cancer cell lines are individualistic. In conclusion, MLS128 inhibited the cell growth in all three colon cancer cell lines examined, and the 110kDa GP has been identified as the MLS128 binding receptor. Although their cell growth is in part IGF-I dependent, growth inhibitory effects of MLS128 are not likely depending on IGF actions, suggesting that MLS128 inhibits colon cancer growth via other growth signaling pathways.

### References

1. Numata Y *et al.* A monoclonal antibody directed to Tn antigen. *Biochem Biophys Res Commun* 1990; 170:981-985.
2. Nakada H *et al.* Epitopic structure of Tn glycoprotein A for an anti-Tn antibody (MLS128). *Proc Natl Acad Sci USA* 1993; 90:2495-2499.
3. Osinaga E *et al.* Analysis of the fine specificity of Tn-binding proteins using synthetic glycopeptide epitopes and a biosensor based on surface plasmon resonance spectroscopy. *FEBS Lett* 2000; 469:24-28.
4. Matsumoto-Takasaka A *et al.* Surface plasmon resonance and NMR analyses of anti Tn-antigen MLS128 monoclonal antibody binding to two or three consecutive Tn-antigen clusters. *J Biochem* 2012; 151:273-282
5. Morita N *et al.* Inhibition of cancer cell growth by anti-Tn monoclonal antibody MLS128. *Biosci Trends* 2009; 3:32-37.

## **6r, a novel oxadiazole analogue of ethacrynic acid, inhibits proliferation of human cancer cells *in vitro* and tumour xenografts *in vivo* by induction of cell apoptosis**

Zhang Peng, Jinhua Chen, Yanna Cheng, Guisen Zhao, Xiuli Guo\*

*Department of Pharmacology, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, Jinan, China.*

This study investigated the *in vitro* and *in vivo* antitumor effects of 5-[2,3-Dichloro-4-(2-methylene-1-oxobutyl) phenoxy-methyl]-3-methyl-1,2,4-oxadiazole (6r), a novel ethacrynic acid (EA) derivative. The *in vitro* effect of 6r on cell proliferation of a panel of human tumor cell lines was assessed using MTT assay and the *in vivo* effect was determined with an SW620 xenografts nude mice model. The effect of 6r on expressions of GST P1-1 and apoptosis-related proteins were measured by western blotting and the effect on cell apoptosis was analysed by Hoechst 33258 nuclear staining as well as by cell surface staining annexin V staining. The effect on cell cycle was assessed by flow cytometry. 6r inhibit proliferation of a range of human cancer cells *in vitro* and growth of tumour xenografts *in vivo*. The anti-proliferative effect of 6r is associated with cell apoptosis as a result of increased ratio of cellular Bax/bcl-2 expression and subsequent cytochrome c and caspase-3 activation. Unlike EA, 6r did not show any influence on cellular GST P1-1 expression and its anti-proliferative action was associated with cell cycle arrest in S phase. 6r inhibits cancer cell proliferation by induction of cell apoptosis but not regulating GST P1-1. 6r has the potential to be developed as a chemotherapeutic agent.

**Keywords:** 6r, Human cancer cell, glutathione S-transferase P1-1, Xenografts, bcl-2/Bax-CytC-caspase-3 mitochondrial pathway



## A potential host-directed therapeutic target against HCV infection elucidated by RNA interference screen

Ri Sho<sup>1</sup>, Xuhong Zhang<sup>2</sup>, Hisayoshi Watanabe<sup>3</sup>, Takafumi Saito<sup>3</sup>, Rika Ishii<sup>3</sup>, Yoshiyuki Ueno<sup>3</sup>, Seiji Hongo<sup>4</sup>, Akira Fukao<sup>1</sup>

1. Department of Public Health.
2. Department of Biochemistry and Molecular Biology, Yamagata University Graduate School of Medical Science.
3. Department of Gastroenterology.
4. Department of Infectious Diseases, Yamagata University Faculty of Medicine.

Hepatitis C virus (HCV) infection is a major public health problem and one of the most important causes of liver diseases such as chronic hepatitis, cirrhosis, and liver cancer worldwide. Current HCV therapy is limited in its efficacy although recently approved HCV protease inhibitors have significantly improved the standard treatment based on a combination of peginterferon and ribavirin. Development of an alternative approach that targets host factors required for the HCV life cycle is thus needed. In an attempt to identify novel host factors involved in HCV infection, we performed a small-scale RNA interference (RNAi) screen in Huh7.5.1 hepatoma cells that support the efficient replication of HCV *in vitro*. RNAi screen of 66 known innate immune-related genes showed that two siRNAs against IkBb and gene Xm (name hidden due to an unpublished study) reduced HCV infection by 50% or more whereas four siRNAs enhanced viral infection by 1.5-fold in Huh-7.5.1 cells. Further loss-of-function analysis revealed that either siRNA silencing or mAb blocking of Xm resulted in a dose-dependent inhibition of HCV replication, suggesting that Xm plays an important role in HCV infection. Moreover, gain-of-function analysis found that over-expression of Xm not only enhanced the viral attachment but also increased the virus production, implying that this host factor may facilitate HCV infection *in vitro* through mediating the viral entry. In conclusion, our study identified a novel host cellular factor required for HCV infection, which should ultimately contribute to develop a host-directed antiviral therapy. In addition, our research also indicated that RNAi screening is an extremely powerful tool for exploring virus-host interactions and elucidating the target genes applicable for the design of new anti-viral drugs.

## **Association of tumor suppressor p53 gene Arg72Pro polymorphism with lung cancer and control subjects in Bangladeshi population**

Md. Aftab Uddin Ph.D.

*Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka 100, Bangladesh, (E-mail: draftabu@gmail.com).*

Cancer is a disease in which cell growth and division are unregulated. This lack of regulation is due to mutations in genes whose protein products are involved in the control of the cell cycle. Humans of all ages develop cancer and a wide variety of organs are affected. The incidence of many cancer increases with the age, so that as people live longer, more will develop the disease. In Bangladesh prevalence of lung cancer is highest in Male. Population-based data on cancer can differ. A recent WHO study estimated that there are 49,000 oral cancer, 71,000 laryngeal cancer and 196,000 lung cancer cases in Bangladesh among those aged 30 years or above in Bangladesh (as of 2004). From the population based study by SH Wild showed that, lung cancer deaths were higher in men than women for all groups, particularly for people born in Bangladesh, the Middle East and the West Indies (Wild et al., 2006). Besides physical and chemical carcinogens mutations in the tumor suppressor gene like p53 play a important role in lung cancer progression (Kawajiri *et al.*1993). We study the association Arg72Pro polymorphism at codon 72 of p53 gene in lung cancer patient and healthy control people of Bangladesh, identified whether homozygous mutant genotype of p53 gene is associated with the severity of lung cancer, and identified the correlation between clinical-histopathological parameters and genotypes of the samples by statistical analysis.

### *References*

1. SH Wild, CM Fischbacher, A Brock, C Griffiths and R Bhopa. Mortality from all cancers and lung, colorectal, breast and prostate cancer by country of birth in England and Wales, 2001-2003. *British Journal of Cancer*. 2006; 94:1079-1085.
2. Kawajiri, K., Nakachi, K., Imai, K., Watanabe, J., and Hayashi, S. I. Germline polymorphism of *p53* and *CYP1A1* genes involved in human lung cancer. *Carcinogenesis*. 1993; 14:1085-1089.

## Design, synthesis and biological evaluation of thiazolidine derivatives as potential anti-tumor agents

Hao Fang, Guizhi Xiao, Shaolei Wu, Yutao Wang, Gang Wang

Department of Med Chem, School of Pharmacy, Shandong University, China

B-cell lymphoma-2 (Bcl-2) family proteins are the key regulators of the intrinsic pathway of apoptosis. The antiapoptotic proteins of Bcl-2 family proteins, such as Bcl-2 and Bcl-xL, are over-expressed in many tumor cells and lead to the drug resistance in chemotherapy. Therefore, Bcl-2 proteins inhibitors can promote tumor cell apoptosis and has the potential application to overcome drug-resistant in cancer chemotherapy. Currently, many small molecules have been identified as Bcl-2 inhibitors and some of them were investigated in clinical trials.<sup>1</sup>

It is well known that thiazolidone derivatives exhibit various pharmacological activities and has been used for developing inhibitors of aldose reductase, translation initiation and PRL-3. In recent decade, some 2-thio-thiazolidone derivatives, such as BHI-3 and WL-276, have been reported to be potent Bcl-2 inhibitors<sup>2-3</sup>. Our previous studies suggest that different substitution in the WL-276 side chain can significantly affect the binding affinity. In our on-going research work, structure modification on 2-thio-4-thiazolidone have been carried out based on the replacement of side chain and scaffold hopping strategy. According to the preliminary biological evaluation results, the modification of the side chain on 2-thio-thiazolidone also can increase the binding affinities, which indicated that the optimization work in our research would be favor to find the efficient and drug-like novel small molecule Bcl-2 inhibitors in the future.

### Reference

1. Youle R, Strasser A. Nat. Rev. Mol. Cell. Biol. 2008; 9:47-59.
2. Degtarev A, Lugovskoy A, Cardone M. Nat. Cell. Biol. 2001; 3:173-182.
3. Xing C, Wang L, Tang X. Cancer Res. 2008; 68:4377-4383.

**Acknowledgement:** Financial support from National Natural Science Foundation of China (21172133) is gratefully acknowledged.

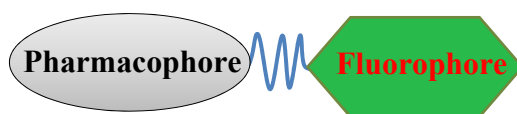
## Fluorescent probes for $\alpha_1$ -adrenergic receptor imaging

Wenhua Li, Lupei Du, Minyong Li\*

*Department of Medicinal Chemistry, School of Pharmacy, Shandong University, Shandong 250012, Chin, (E-mail: mli@sdu.edu.cn).*

$\alpha_1$ -adrenergic receptors (ARs), as the crucial members of G protein-coupled receptors (GPCRs), often mediate various physiological responses of the sympathetic nervous system. So far,  $\alpha_1$ -ARs have at least three major subtypes,  $\alpha_{1A}$ ,  $\alpha_{1B}$  and  $\alpha_{1D}$ , which carry out prominent roles in human various tissues and organs. However, to date it is strenuous either to establish the distribution of each  $\alpha_1$ -adrenoceptor subtype in various tissues and organs, or to define the pharmacological properties mediated by each one in the different species. Fortunately, with the rapid development of fluorescent labeling method for the receptors pharmacological research, the medicinal chemists have designed a number of fluorescent ligands for tagging and visualizing the receptors to obtain the functional information. In this research we designed and synthesized a series of the small-molecule fluorescent probes of  $\alpha_1$ -adrenergic receptor, as shown in Figure 1, which mainly contain two parts: the pharmacophore that can be bound to  $\alpha_1$ -adrenergic receptors, and the fluorophore that can be used to make the receptors visualization. Moreover, the biological evaluation displays that the small-molecule fluorescent probes have excellent fluorescence imaging potential, which can significantly label the  $\alpha_1$ -adrenergic receptor in living cells.

**Figure 1**



## Oral presentation

### Session III Drug Discoveries: natural bioactive agents and drug evaluation

#### Anti-hepatitis B virus activities of cinobutacini and related compounds in HepG2.2.15 cells

Jufeng Xia<sup>1</sup>, Xiaoyan Cui<sup>1</sup>, Yoshinori Inagaki<sup>1</sup>, Norihiro Kokudo<sup>1</sup>, Bo Gao<sup>2</sup>, Wei Tang<sup>1,\*</sup>

1. Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.
2. Anhui Jinchan Biochemical Co., Ltd, Huaibei, China.

Hepatitis B virus (HBV) infection is a major threat to public health worldwide and is the leading cause of chronic hepatitis around the world. However, there are limited therapeutic options for the millions of chronically infected individuals. Therefore, the discovery and development of novel antiviral drugs for the treatment of HBV is in urgent need. Traditional Chinese medicines (TCMs), widely used to treat hepatitis B in China and many other parts of the world, may offer candidates with special antiviral characteristics. Cinobufacini, the aqueous extract of the skin of *Bufo bufo gargarizans* Cantor, has been extensively used in clinics to treat malignant tumors, chronic hepatitis B, etc. The aim of present study was to examine the anti-HBV activities of cinobutacini and its pure compounds (bufalin and cinobufagin) in the human HBV-transfected liver cell line HepG2.2.15. The HBsAg, HBeAg, and HBcrAg concentrations in cell culture medium were determined by using chemiluminescent enzyme immunoassay after HepG2.2.15 cells were respectively treated with cinobutacini, bufalin, and cinobufagin. HBV DNA in culture medium was analyzed by the transcription-mediated amplification and HBV mRNA in cells was determined by real-time PCR. Cinobutacini at 1 µg/mL effectively inhibited the secretion of HBsAg, HBeAg, and HBcrAg and was more potent than the positive control lamivudine (100 µg/mL). Moreover, the inhibitory activity of cinobutacini on secretion of three HBV antigens was promoted time-dependently. Bufalin and cinobufagin slightly inhibited the secretion of HBV antigens. Treatment with cinobutacini, bufalin, or cinobufagin had no anti-HBV effect on DNA in cell culture medium. Consistent with the HBV antigen reduction, HBV mRNA was markedly depressed when HepG2.2.15 cells were respectively treated with cinobutacini, bufalin, and cinobufagin, as compared to the control. These findings should enlighten that cinobutacini possesses potent anti-HBV activity and its activity on inhibition of HBV antigen secretion is attributed to the specific depression of HBV mRNA expression.

## **Bufalin and cinobufagin induce apoptosis in HepG2 cells through Fas- and mitochondria-mediated pathways**

Fanghua Qi<sup>1,2</sup>, Yoshinori Inagaki<sup>2</sup>, Norihiro Kokudo<sup>2</sup>, Anyuan Li<sup>1</sup>, Wei Tang<sup>2,\*</sup>

1. Department of Traditional Chinese Medicine, Shandong Provincial Hospital, Shandong University, Ji'nan, Shandong, China;

2. Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

Hepatocellular carcinoma (HCC) is among the five most frequent cancers worldwide. This disease has few effective chemotherapeutics. Therefore, development of new therapeutic agents and more effective therapies for the treatment of HCC are in urgent need. Lately, traditional Chinese medicines and their effective compounds were reported to play important role in HCC therapy, especially bufalin and cinobufagin, the active compounds of Chan Su. Although bufalin and cinobufagin have been reported to induce apoptosis of various cancer cells by activation of activator protein-1 (AP-1), NF- $\kappa$ B, T lymphoma invasion and metastasis gene 1 (Tiam1), the c-Jun N-terminal protein kinases (JNKs), Rac1, mitogen-activated protein kinase (MAPK) and Bax and inhibition of Bcl-2 and c-myc, the detailed molecular mechanisms of apoptosis induced by bufalin and cinobufagin in HCC remain unclear. In our study, the details of apoptotic signaling pathways, both the mitochondria- and Fas-mediated pathways, are shed light on. We found bufalin and cinobufagin induced striking changes in apoptotic morphology and significantly increased the proportion of apoptotic cells. The apoptosis of HepG2 cells was induced by an increase in Fas and Bax expression, a decrease in Bcl-2 expression, disruption of the mitochondrial membrane potential, release of cytochrome c, activation of caspase-3, -8, -9 and -10, and cleavage of Bid and poly (ADP-ribose)polymerase (PARP), which suggested that the two pathways worked at the same time. Moreover, for further validating the caspase activation during bufalin- and cinobufagin-induced apoptosis, caspase-3 inhibitor Z-DEVD-FMK, caspase-8 inhibitor Z-IETD-FMK, caspase-9 inhibitor Z-LEHD-FMK and caspase-10 inhibitor Z-AEVD-FMK were employed. As a result, bufalin- and cinobufagin-induced apoptosis was restrained by these inhibitors and particularly by caspase-3 and -10 inhibitors. In conclusion, bufalin and cinobufagin induced apoptosis of HepG2 cells through both mitochondria- and Fas-mediated signaling pathways and the cross-talk between the two pathways might exist. These findings should enlighten on the further exploration of potential of bufalin and cinobufagin for use in HCC therapy.

## Use of silkworm infection model to identify novel therapeutically effective antibiotic, kaikosin E

Atmika Paudel<sup>1</sup>, Hiroshi Hamamoto<sup>1</sup>, Makoto Urai<sup>1</sup>, Jie Su<sup>1</sup>, Keiko Kataoka<sup>2</sup>, Kazuhisa Sekimizu<sup>1</sup>

1. Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan;

2. Genome Pharmaceuticals Institute, Co. Ltd., 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.

We established silkworm (*Bombyx mori*) infection model for the evaluation of therapeutic effects of antimicrobial agents. We are using this model for the screening of natural as well as synthetic products. Use of the silkworm infection model is not only efficient for identifying therapeutically effective antibiotics at an early stage of screening, but also for reducing the time, effort, and cost associated with conventional screening methods. We showed the ED<sub>50</sub> values of clinically used antibiotics in silkworms were similar to those in mammalian model. On the course of screening natural products against *Staphylococcus aureus* infection, we identified a novel antibiotic, kaikosin E from a soil dwelling lysobacter species. Purification was achieved with organic solvent extraction, ODS open column chromatography and reverse phase HPLC. Five milligrams of kaikosin E was purified from 1.2 L of broth. Structure of kaikosin E was revealed by mass spectrometer and NMR analysis. It has a large molecular mass of 1617 and is a cyclic lipopeptide containing 12 amino acid residues and a short fatty acid chain. Kaikosin E showed antimicrobial activity against Gram-positive bacteria, including methicillin resistant *S. aureus*, with MIC values ranging from 0.13 to 25 µg ml<sup>-1</sup>. Interestingly, its ED<sub>50</sub> value in the mouse infection model was 0.6 mg kg<sup>-1</sup>, indicating that it is more potent than vancomycin (ED<sub>50</sub>: 1.6 mg kg<sup>-1</sup>). Mice injected with 400 mg/kg of kaikosin E showed no signs of acute toxicity suggesting that the acute toxic dose of kaikosin E is more than 400 mg/kg. Its potent therapeutic effectiveness and low toxicity suggest that it has a strong potential to be a candidate for clinical application. Thus, the silkworm infection model is a useful model for identifying therapeutically effective novel antibiotics.

## Identification and expression analysis of key enzymes of the terpenoids biosynthesis pathway of a liverwort *Plagiochasma appendiculatum* by EST analysis

Aixia Cheng, Lei Wang, Yi Sun, Hongxiang Lou

*School of Pharmaceutical Sciences, Shandong University, No. 44 West Wenhua Road, Jinan, China.*

Liverworts are known to be a rich source of terpenoids and phenolic compounds whose biochemical and molecular biosynthesis properties are not well understood. To evaluate the biosynthetic processes of such compounds found in a liverwort *Plagiochasma appendiculatum*, a total of 5024 clones were sequenced from a normalized cDNA library from leaves of *P. appendiculatum*. This produced 4384 high-quality ESTs with a mean length of 550 bp. Cluster analysis indicated the presence of 704 contigs and 2720 singletons, generating 3424 unique sequences. A total of 1180 sequences were functionally classified using gene ontologies (GO). Based on homology to sequences present in GenBank, our EST collection was found to contain orthologs for known prenyl transferases and for genes involved in the 2-C-methyl-d-erythritol 4-phosphate (MEP) and the mevalonic acid (MVA) pathways, both of which are involved in terpenoid biosynthesis. Informatic analysis revealed that all of the genes involved in terpenoid biosynthesis showed extensive homology with tracheophyte genes. Phylogenetic analysis indicated that the topology of the phylogenetic trees constructed using genes MEP, DXR and HMGS was in good agreement with traditional taxonomic classification, but the one constructed using gene FPS was not. Real-time PCR showed that the expression level of these genes was enhanced after the MeJA treatment, as in vascular plants.

**Keywords:** *Plagiochasma appendiculatum*; terpenoid biosynthesis, EST sequence; phylogenetic analysis; gene expression



---

## Separation and absolute configuration assignment of two diastereomeric pairs of enantiomers from *Lobelia chinensis* by high-performance liquid chromatography with a circular dichroism detector

Shuang Yang, Dongmei Ren\*

Department of Natural Products Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44 Wenhuxi Road, Jinan, PR China.

*Lobelia chinensis* is one species of genus *Lobelia*, family Campanulaceae, which distributed widely in East Asia. The aerial parts of the plant have been used as diuretic, antidote and hemostat in traditional Chinese medicine. By using silica gel chromatography, Sephadex LH-20 chromatography, RP-18 chromatography and semi-preparative HPLC, the current work led to the isolation of an unprecedented compound, named lobechninoid. This compound was elucidated as a conjugate of lignan and alkaloid by spectroscopic methods. Due to the equipment of the two stereogenic centers, lobechninoid was chiral. By using chiral HPLC-CD, lobechninoid was determined as mixtures of two diastereomeric pairs of enantiomers. The on-line CD spectra of the four stereoisomers were obtained and used for the assignment of the absolute configurations successfully, which indicated HPLC-CD technique was a reliable stereoanalytical tool for natural products.

## Antimicrobial mechanism of a novel compound, Kaikosin E

Jie Su<sup>1</sup>, Hiroshi Hamamoto<sup>1</sup>, Atimika Paudel<sup>1</sup>, Makoto Urai<sup>1</sup>, Keiko Kataoka<sup>2</sup>, Kazuhisa Sekimizu<sup>1</sup>

1. Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan;

2. Genome Pharmaceuticals Institute, Co. Ltd., 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

Kaikosin E is a novel cyclic lipopeptide antimicrobial agent screened from a lysobacter strain using silkworm infection model. It exhibits good antibiotic traits such as obvious therapeutic effect in mouse infection model with low toxicity and potent antimicrobial activity against gram-positive pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA). The objective of this study was to elucidate the antibacterial mechanism of Kaikosin E. Kaikosin E exerted bactericidal activity against *S. aureus* in short-term (within 1min). Cell lysis of *S. aureus* was observed after treatment with Kaikosin E. Kaikosin E showed inhibitory effect on biosynthesis of DNA, RNA, protein and cell wall. Addition of Kaikosin E to culture medium of *S. aureus* rapidly caused depolarization of membrane potential. Furthermore, treatment with 100µg/mL (25-fold MIC against *S. aureus*) of Kaikosin E had no lysing effect on sheep red blood cell, suggesting that this membrane damaging effect is specific to bacteria. Based on these finding, the proposed mechanism is that Kaikosin E interacts with cell membrane to disrupt membrane integrity, following with membrane depolarization and cell death. The rapid bactericidal activity indicates the mechanism of Kaikosin E is novel from other clinically used antibiotics.

## Antiproliferative and Antimicrobial activities of alkybenzoquinone derivatives from *Ardisia kivuensis*

Dzoyem J. Paul<sup>1</sup>, Ndontsa B. Laure<sup>2</sup>, Santosh K. Guru<sup>3</sup>, Hamamoto H.<sup>4</sup>, Matsumoto Y<sup>4</sup>, Inshad A. Khan<sup>5</sup>, Saxena K. Ajit<sup>3</sup>, Ram A. Vishwakarma<sup>6</sup>, Sekimizu K.<sup>4</sup>, Tane Pierre<sup>2</sup>.

1. Department of Biochemistry; Faculty of Science, University of Dschang, Cameroon;
2. Department of Chemistry, Faculty of Science, University of Dschang, Cameroon;
3. Cancer Pharmacology Division, Indian Institute of Integrative Medicine, Jammu, India;
4. Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan;
5. Clinical Microbiology Division, Indian Institute of Integrative Medicine, Jammu, India;
6. Medicinal Chemistry Division, Indian Institute of Integrative Medicine, Jammu, India.

Four naturally occurring alkybenzoquinone derivatives, namely ardisiaquinone N (1), ardisiaquinone J (2), ardisiaquinone K (3) and a mixture of ardisiaquinone P (4) and K (3) isolated from the MeOH extract of the stem barks and leaves of *Ardisia kivuensis* were investigated *in vitro* for cytotoxicity against a panel of human cancer cell lines including lung A549 adenocarcinoma, breast carcinoma MCF-7, prostate carcinoma PC-3, cervical carcinoma HeLa and acute monocytic leukemia cell line THP-1. In addition, the antimicrobial activity was evaluated against three gram negative, three gram positive bacteria and three fungi. Minimum Inhibitory Concentration (MIC) was determined using the broth micro-dilution assay. Tumor cells growth inhibition was determined by sulphorhodamine B (SRB) assay. To further determine whether the THP-1 growth inhibitory activity of compounds was related to apoptosis induction, sub-diploid DNA fraction was measured by flow cytometry. All the compounds tested showed MIC values >10 µg/ml hallmark of moderate antimicrobial activity. The four compounds exhibited remarkable antiproliferative activity against the leukemia cell line TPH-1 with IC<sub>50</sub> inhibition values between 2-2.1 µg/ml. The cytotoxic activity found to be related to apoptosis induction. These findings suggest that natural compounds herein studied are interesting potential candidates for the development of new therapeutic agents especially against leukemia.

## Bioactive chemicals from *Curcuma xanthorrhiza*

Peihong Fan, Chunmei Zhang

*Department of Natural Product Chemistry, Key Lab of Chemical Biology of Ministry of Education, Shandong University.*

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of senile plaques composed of  $\beta$ -amyloid peptide ( $A\beta$ ) strongly associated with the loss of cognition and memory. The most dramatic abnormalities noted in AD brains are associated with the cholinergic system. Acetylcholinesterase (AChE) plays a key role in the regulation of the cholinergic system by terminating signaling at cholinergic synapses, and hence, inhibition of AChE has emerged as one of the most promising strategies for the treatment of AD. In our endeavor to screen natural acetylcholinesterase inhibitors from herbs, Extract of *Curcuma Xanthorrhiza* was found to be active both by TLC bioautograph technique and Ellman test. Its chemical constituents were further studied. Except curcumin and its derivatives, four sesquiterpenes include zederone, curcumadione, curcumenone and a guaianolide Guai-1(10),3,5,7(11),8-pentaen-2-on-11,8 -olide were isolated and identified, as well as  $\beta$ -sitosterol and stearic acid. The guaianolide was first reported from the *curcuma* species. But the isolated sesquiterpenes showed weak AChE inhibitory activity *in vitro* comparing with the volatile oil of *C.xanthorrhiza*. GC-MS was further used to identify the chemicals of volatile oil. The *in vivo* of AChE inhibitory activity is being investigated. *C. xanthorrhiza* is one ingredient of curry. Some epidemiological studies provided evidence that curry diet is related to the relative low rate of AD in Indian people. Curcumin has been reported to have anti- $A\beta$  aggregate activity. This work revealed that AChE inhibition maybe another effect of *C. xanthorrhiza* to prevent and treat AD.

## Antimetastatic and antiangiogenic activities of sulfated polysaccharide of *Sepiella maindroni* ink

Aizhen Zong<sup>1</sup>, Ting Zhao<sup>1</sup>, Yan Zhang<sup>1</sup>, Xinlei Song<sup>1</sup>, Yikang Shi<sup>2</sup>, Hongzhi Cao<sup>2</sup>, Chunhui Liu<sup>1,2</sup>, Xianjun Qu<sup>2,3</sup>, Fengshan Wang<sup>1,2,\*</sup>

1. Key Laboratory of Chemical Biology of Natural Products (Ministry of Education), Institute of Biochemical and Biotechnological Drugs, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, Shandong, P.R. China;
2. National Glycoengineering Research Center, Shandong University, Jinan 250012, Shandong, P.R. China;
3. Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University, Jinan 250012, Shandong, P.R. China

Our previous study demonstrated that SIP-SII, a sulfated *Sepiella maindroni* ink polysaccharide, suppressed the invasion and migration of cancer cells via the inhibition of the proteolytic activity of matrix metalloproteinase-2 (MMP-2). Therefore, this study investigated the anti-metastatic effect of SIP-SII *in vivo*. SIP-SII (15 and 30mg/kg·day) markedly decreased B16F10 pulmonary metastasis in mice models by 85.9% and 88.0%, respectively. Immunohistochemistry showed that SIP-SII decreased the expression of the intercellular adhesion molecule 1 (ICAM-1) and basic fibroblast growth factor (bFGF) in lung metastasis nodules. In addition, SIP-SII inhibited neovascularization in chick chorioallantoic membrane assay at 0.08-2mg/mL. In the *in vitro* experiments, SIP-SII (0.8-500µg/mL) significantly decreased the protein and mRNA expression of ICAM-1 and bFGF in SKOV3 and EA.hy 926 cells, respectively. These results suggested that SIP-SII might suppress melanoma metastasis via the inhibition of the tumor adhesion mediated by ICAM-1 and the angiogenesis mediated by bFGF, as well as resulting in depression of the invasion and migration of carcinoma cells.

## Poster presentation

### Session I Cancer treatment and drug discoveries

#### The multifunctional nanoscaled delivery system for codelivery of nucleic acids and chemotherapeutics

Chunxi Liu, [Na Zhang](#)

Department of Pharmaceutics, School of Pharmaceutical Science, Shandong University, 44 West culture Road, Ji'nan 250012, Shandong Province, China.

Codelivery of different therapeutics has a potential to efficaciously treat human diseases via their synergistic effects. Activable therapeutic tools at the nanoscale are suitable platforms for codelivery of different therapeutics, including nucleic acids and chemotherapeutics. We have recently developed a multifunctional nanoscaled delivery system simultaneously applied with tumor-targeted, pH-triggered and codelivery strategies. In this study, DOX-HZN-PEI-PEG-TAT was first synthesized and complexed with pDNA to form DOX loaded-TAT modified polyion complex micelles (PIC). Next, sulfamerazine (SA)-PEG-NGR (SA, pKa = 7.0) was synthesized and attracted on the surface of PIC to obtain NGR modified PIC micelles (targeted PIC micelles, TPIC). SA is weakly acidic due to the readily ionizable hydrogen atom in the amide bond in water. Above the pKa, SA has a negative charge, while it is neutral below the pKa. Therefore, at physiological pH (pH = 7.4), the TPIC could be constructed by the electrostatic interaction between the positive charged core of PIC and negatively SA. Meanwhile, the steric hindrance created by the long PEG spacer was expected to shield the surface-attached TAT to reduce its associated side effects on normal tissue. While, at tumour site (pH < 7.0), SA-PEG-NGR could be dissociated from the surface of PIC and TAT was exposed to facilitate the tumour cellular uptake. The resultant DOX-HZN-PEI-PEG-TAT conjugates had a drug loading of  $8.01 \pm 1.22\%$ . The results of in vitro DOX release confirmed that the DOX release via pH-dependent hydrolysis of the hydrazone bond, which showed lower release rate at pH 7.4 and higher rate pH 5.0. In addition, DOX-HZN-PEI-PEG-TAT exhibited higher cell cytotoxicity at pH 5.0 than pH 7.4 ( $p < 0.05$ ) and higher fluorescence intensity in cells at pH 5.0 was shown than that at pH 7.4. The obtained PIC and TPIC displayed nearly spherical shape and no aggregation was observed. The sizes were  $187.6 \pm 4.3\text{nm}$  and  $221.2 \pm 6.8\text{nm}$ , respectively. The zeta potentials were  $20.5 \pm 2.4\text{mV}$  and  $-10.9 \pm 1.3\text{mV}$ , respectively. The results of in vitro cell studies indicated that both PIC and TPIC could successfully transfect HUVEC, MCF-7 and SKOV-3 cells. Compared with PIC, TPIC exhibited higher transfection efficiency in CD13<sup>+</sup> cells than CD13<sup>-</sup> cells. More importantly, both PIC and TPIC were able to simultaneously accomplish intracellular drug delivery and gene transfection, indicating that the co-delivery is a cooperative process. All in all, the novel multifunctional nanoscaled delivery system is promising carrier for the codelivery of nucleic acids and chemotherapeutic agents.

**Keywords:** Codelivery, multifunctional nanoscaled delivery system, doxorubicin; gene delivery, polyion complex micelles

---

## Effects of anti-insulin-like growth factor-I receptor (IGF-IR) antibodies with or without the ER-retention signal KDEL on cancer cell growth

Yuming Guo, Noriyuki Yuasa, Yoko Fujita-Yamaguchi

*Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, 259-1292, Japan.*

Recombinant antibody consisting of the single-chain variable fragment (scFv) of 1H7 monoclonal antibody against IGF-IR and human IgG<sub>1</sub> Fc domain, scFv-Fc, has been found to exhibit inhibitory effects on breast cancer growth *in vitro* and *in vivo* [Li *et al. Cancer Immunol. Immunother.* 49, 243, 2000; Sachdev *et al. Cancer Res.* 63, 627, 2003]. In this study, we constructed an intrabody-type of 1H7 scFv-Fc which should have more potent growth inhibitory effects than 1H7 scFv-Fc. pCIneo expression vectors encoding 1H7 scFv-Fc-KDEL and 1H7 scFv-Fc as well as Fc-KDEL and Fc were constructed, and then introduced into MCF-7 breast cancer cells. Intracellular expression of 1H7 scFv-Fc KDEL and Fc-KDEL was confirmed by ELISA and immunofluorescent microscopy. MCF-7 cell growth was significantly inhibited by transfection of pCIneo-1H7 scFv-Fc-KDEL when compared to that of pCIneo-1H7 scFv-Fc-transfected cells. Whether or not the intrabody inhibits cell growth by retaining IGF-IR inside cells is under investigation.

## Chitosan-based Intelligent Charge-reversal System for High Efficiency Delivery of Paclitaxel

Yufeng Ma<sup>1,2</sup>, Piping Lv<sup>2</sup>, Zhanguo Yue<sup>2</sup>, Wei Wei<sup>2,\*</sup>, Guanghui Ma<sup>2</sup>, Rong Yu<sup>1,\*</sup>

1. Key Laboratory of Drug Targeting and Drug Delivery System, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu 610041, China;
2. State Key Lab. Biochem. Eng., Institute of Process Engineering, Chinese Academy of Science, Beijing 100190, China.

After modification by tris(2-aminoethyl) amine (TAEA) and 2,3-dimethylmaleic anhydride (DMMA), chitosan nanoparticles were endowed with intelligent charge-reversal ability. The fabricated system (CNP:TAEA:DMMA<sup>-</sup>) was then loaded with insoluble anticancer drug paclitaxel (PTX), and drug delivery efficiency was tested *in vivo*. Results showed that, PTX-CNP:TAEA:DMMA<sup>-</sup> was negatively charged (-11 mV) at normal tissue circumstance (pH 7.4) and rarely internalized by J774.1 cells, exhibiting stealth effect to macrophages. Once arriving at the cancer site, those nanoparticles could intelligently transform to positive charge (+5 mV) by the low-pH microenvironment in tumor tissue, which could improve tumor cell uptake and accordingly increase the drug concentration at the lesion site. Compared with commercial PTX formulation, this intelligent charge-reversal system possessed superior biocompatibility and better cytotoxicity (the value of half inhibitory concentration was decreased to 4.09 µg/mL from 11.3 µg/mL), holding a great potential as efficient anti-cancer drug carrier.

**Key words:** charge-reversal, paclitaxel, drug carrier, chitosan nanoparticle



## Chemoprevention of intestinal adenomatous polyposis by acetyl-11-keto-beta-boswellic acid (AKBA) in $APC^{Min/+}$ mice

Huiping Liu<sup>1</sup>, Zuhua Gao<sup>2</sup>, Shuxiang Cui<sup>3</sup>, Yan Wang<sup>3</sup>, Baoying Li<sup>4</sup>, Hongxiang Lou<sup>1\*</sup>, Xianjun Qu<sup>1\*</sup>

1. Department of Pharmacology, Key Laboratory of Chemical Biology, School of Pharmaceutical Sciences, Shandong University, Jinan, China;
2. Department of Pathology and Laboratory Medicine, University of Calgary and Calgary Laboratory Services, Calgary, Alberta, Canada;
3. Department of Pharmacology, Institute of Materia Medica, Shandong Academy of Medical Sciences, Jinan, China;
4. Qilu Hospital of Shandong University, Jinan, China.

Acetyl-11-keto-beta-boswellic acid (AKBA) is the derivative of boswellic acids that are the effective components of gum resin of *Boswellia serrata*. AKBA has been used as an adjuvant medicine for treatment of inflammatory diseases. In this study, we aimed to evaluate the efficacy of AKBA as a chemopreventive agent against intestinal adenomatous polyposis in  $APC^{Min/+}$  mouse model.  $APC^{Min/+}$  mice were given AKBA by p.o. gavage for 8 consecutive weeks. Mice were sacrificed and the number, size and histopathology of intestinal polyposis were examined under a microscope. AKBA effectively prevented the formation of adenomatous polyposis, showing a reduction of polyp's number, size and appearance. AKBA inhibited polyp number by 48.9% in small intestine and 60.4% in colon. More prominent AKBA effect was observed in the prevention of colonic adenocarcinoma. The number of large colonic polyposis was reduced by 77.8%. Histopathologic analysis indicated that the ulcerated shapes of intestinal polyps were obviously reduced and numbers of anaplastic cells were largely decreased in intestinal villus of the AKBA-treated mice. In the term of study, AKBA was well tolerated by mice with no obvious signs of toxicity to animal. The assays of immunohistochemical staining, western blotting and enzyme-linked immunosorbent assay (ELISA) indicated that the prevention of intestinal polyps by AKBA was due to its activities of anti-proliferation, apoptotic induction, antiangiogenesis, and anti-inflammation. AKBA might exert its chemopreventive action through inhibition of Wnt/ $\beta$ -catenin and NF- $\kappa$ B/COX-2 signaling pathways. Our findings suggested that this naturally occurring food component could be a promising regimen in chemoprevention against intestinal tumorigenesis.

## **Inhibition of peanut agglutinin lectin-induced cancer cell growth by anti-Tn monoclonal antibody MLS128**

Keito Iijima<sup>1</sup>, Fumie Oura<sup>1</sup>, Yukiko Yajima<sup>1</sup>, Hiroshi Nakada<sup>2</sup>, Yoko Fujita-Yamaguchi<sup>1</sup>

*Tokai University School of Engineering, Department of Applied Biochemistry, Hiratsuka, Kanagawa, 259-1292, Japan;*

*Kyoto Sangyo University, Department of Molecular Bioscience, Kyoto, Kyoto, 603-8555, Japan.*

Peanut agglutinin lectin (PNA) binds to T-antigen (Gal $\beta$ 1-3GalNAc $\alpha$ -Ser/Thr) and is mitogenic for colorectal cancer cell lines. Peanut ingestion causes increased proliferation of rectal epithelia in individuals with mucosal expression of T-antigen. Proliferative effects of PNA on HT29 colon cancer cells involve high molecular weight isoforms of transmembrane glycoprotein CD44v6 and its association with c-Met activation by its natural ligand, hepatocyte growth factor/scatter factor (HGF/SF), and subsequent mitogen-activated protein kinase (MAPK) activation (Singh et al. Glycobiology 2001 & 2006). In this study, we studied effects of PNA on LS180 colon cancer cell growth. Addition of PNA, at 2.5 and 25  $\mu$ g/ml, in serum-free medium containing 0.1% BSA, significantly stimulated the growth of LS180 cells to levels of  $118.0 \pm 0.05$  % and  $123.3 \pm 0.02$  % ( $n = 8$ ), respectively, of that without the PNA treatment on day 3. HGF did not stimulate LS180 cell growth. Growth stimulatory effects of PNA were inhibited by anti-Tn-antigen (GalNAc $\alpha$ -Ser/Thr) MLS128, which suppressed LS180 cell growth in the medium containing 1% FBS (Morita *et al.* Biosci. Trends 2009). Possible interactions between PNA and MLS128 signaling pathways in LS180 cells are under investigation.

## Refolding of scFv antibodies from intrabodies expressed in *E.coli* using lauroyl-L-glutamate

Hiroyuki Kato, Takahito Fukuda, Hajime Sato, Noriyuki Yuasa, Yoko Fujita-Yamaguchi

*Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, 259-1292, Japan.*

**Objectives:** Inclusion bodies accumulated in *Escherichia coli* are widely used for a large-scale production of therapeutic proteins. Isolation and characterization of anti-Tn antigen 4E10 and anti-lacto-N-fucopentaose III 1F12 scFvs from a phage library were previously described [Sakai *et al.* J. Biochem. 147, 809, 2010 and Yuasa *et al.* J. Biol. Chem. 285, 30587, 2010, respectively]. In this study, these 4E10 and 1F12 scFv proteins were purified as inclusion bodies that were accumulated in *E. coli* transformants after induction with IPTG. Inclusion bodies were then subjected to refolding using lauroyl-L-glutamate, a novel amino acid-based detergent [Kodou *et al.* Protein Expr. Purif. 75, 46, 2011].

**Methods:** 4E10 scFv and 1F12 scFv genes were subcloned into pET22b(+) vector, which yielded pET-4E10 and pET-1F12 expression vectors. After induction with 1 mM IPTG at 37°C for 4 h, pET/4E10 or pET/1F12 transformed-bacterial cells were solubilized with B-PER® solution. Insoluble fractions were recovered as inclusion bodies which were washed with 10-fold diluted B-PER® solution, then twice with 100% acetone. The purified inclusion bodies were solubilized in 20 mM phosphate buffer, pH 8.5, containing 5% lauroyl-L-glutamate at a concentration of 30 mg /ml and then diluted 2-fold to a final concentration of 2.5% lauroyl-L-glutamate. To this solution, 100 mM dithiothreitol (DTT) was added to a final concentration of 1.2 mM. The solution was incubated at 37°C for 30 min. After the concentration of lauroyl-L-glutamate was lowered to 1%, the sample solution was incubated at 5°C for 30 min, followed by 20-fold dilution with the buffer containing 0.4% lauroyl-L-glutamate, 0.84 M Arg, 1.05 mM GSH and 1.05 mM GSSG. This solution was incubated at 5°C for 17 h, followed by incubation at 23°C for 43 h, and dialyzed against PBS

**Results and Conclusions:** After preparation and cleaning-up of the inclusion bodies, both 4E10 and 1F12 scFv proteins were found to be nearly homogeneous. Starting from 250 ml of *E. coli* cultures, 109.2 mg and 116.8 mg of soluble 4E10 and 1F12 scFv proteins, respectively, were recovered by the new refolding procedures. Determinations of affinity and specificity of the refolded scFv proteins are now in progress.

## **Riccardin D-26 induces apoptosis in KB and the MDR counterpart KB/VCR cells: *In vitro* and *in vivo* studies**

Bin Yue<sup>1</sup>, Cui-Rong Zhao<sup>1</sup>, Huimin Xu<sup>2</sup>, Yuanyuan Li<sup>1</sup>, Hanni Ke<sup>1</sup>, Yi Yuan<sup>1</sup>, Ruiqi Wang<sup>1</sup>, Hongxiang Lou<sup>1\*</sup>, Xianjun Qu<sup>1\*</sup>

1. Department of Pharmacology, Key Laboratory of Chemical Biology, School of Pharmaceutical Sciences, Shandong University, Jinan, China;
2. Faculty of Radiologic Sciences, School of Medicine, Qingdao University, Qingdao, China.

Overcoming the apoptosis deficiency has been verified a promising way to treat multidrug resistance cancer. In the precious study, we have reported that Riccardin D-26 exhibited potential activity against a variety of cancer cells without significant toxicity. Herein, we evaluated the inhibitory effect of Riccardin D-26 on the growth of human oral squamous carcinoma cells KB and its multidrug resistance counterpart KB/VCR cells both *in vitro* and *in vivo*. Riccardin D-26 effectively inhibited cancer cells proliferation as determined by the assays of MTT and clonogenic formation. The inhibitory effect was correlated with apoptosis, which is demonstrated by Annexin-V/PI staining and cleavage of caspase-3/9 and PARP. Further studies indicated that mitochondria mediated intrinsic pathway was involved as determined by JC-1 staining and cytochrome c redistribution. *In vivo* assays showed significant delay of KB and KB/VCR tumor growth in nude mice administrated with Riccardin D-26 while no obvious toxicity was observed. TUNEL assay and western blotting indicated the apoptosis in the tumor tissue. Notably, Riccardin D-26 inhibited cancer growth and induced apoptosis in KB/VCR cells more potently than that of vincristin. In addition, significant changes of phosphorylation levels of MAPK and Akt were detected both in cultured cells and in xenografts. Together, our data suggested that Riccardin D-26 inhibited the MDR cancer growth by triggering apoptosis. Riccardin-26 was thus potential to be used in clinical trial of MDR cancer treatment.

## Screening anti-angiogenic activities of *Sanguisorba* saponins by CAM model

Sanhai Qin\*, Jun Li, Ling Zhou

*Department of Pharmacology, Institute of Materia Medica, Shandong Academy of Medical Sciences, Key Laboratory for Rare & Uncommon Diseases of Shandong Province, Ji'nan 250062, China.*

The roots of *Sanguisorba officinalis* L.(Rosaceae), commonly called 'Di Yu' in Chinese, have been used in traditional medicine for the treatment of hemostasis, burns, scalds, and antipathogen. *S.officinalis* is known to contain triterpenoid saponins and more than 50 triterpenoids have been reported. Our previous pharmacological experiment showed that *S. officinalis* could inhibit tumor both in vitro and in vivo. fourthmore, it could significantly inhibit the neovascularization of chick embryo. The potential medicinal importance and our interest in the bioactive constituents prompted us to investigate the triterpenoid saponins of *S.officinalis*, which resulted in the isolation of a new 19-oxo-18, 19-seco-ursane-type triterpenoid saponin, together with 12 known saponins. The purpose of the present work, therefore, is to screen the anti-angiogenic triterpenoid saponins from *Sanguisorba officinalis*. 7- day-old fertilized white chicken eggs were incubated at 37°C, 3 days later, chick embryos were removed out and placed onto plates for further development. After incubating on the plate for 3 days, carriers were grafted onto chick chorioallantoic membranes (CAM) and treated with *Sanguisorba* saponins in the concentration of 100 µM per egg daily for 48h. On day 8, the number of CAM blood vessel embranchment 5mm around the carrier was counted with a stereomicroscope in 20 magnification observation, and then compared with the NS group (the negative control) to assess whether *Sanguisorba* saponins inhibit the neovascularization of chick embryo. After 48h administration, S-04 and S-06 depressed the angiogenesis in CAM significantly, whose microvessels number was  $23.4 \pm 4.2$  and  $26.8 \pm 5.6$  respectively. Compared with the NS group  $58.6 \pm 7.8$ , the inhibition rate was 60.1% and 54.3% respectively. In conclusion, after screening 13 *Sanguisorba* saponins from *Sanguisorba officinalis*, S-04 and S-06 have anti-angiogenic activities.

**Keywords:** *Sanguisorba* saponins, CAM, anti-angiogenesis

## **Inhibitory effect of CMC on human chronic myeloid leukemia K562 cells and molecular mechanism underlying CMC-induced apoptosis**

Liyan Song, Tian Tian, Qin Zheng, Xianjing Hu, Rongmin Yu\*

*Biotechnological Institute of Chinese Materia Medica, Jinan University, Guangzhou 510632, China.*

Traditional Chinese medicines are a source of alternative cancer therapy and provide a guide for identification of new anticancer constituents. This study investigated the in vitro antitumor activity of *Cordyceps militaris* Constituents (CMC) and the molecular mechanism underlying the apoptosis it induces in human chronic myeloid leukemia K562 cells. The results revealed that CMC triggered growth inhibition in K562 cells with only minor toxicity on a normal human cell line. CMC inhibited the proliferation of K562 cells in a dose- and time-dependent manner, with IC<sub>50</sub> value of  $34.1 \pm 2.0$   $\mu\text{g/ml}$  after 48h incubation. This most likely resulted from the cell cycle arrest at the S phase and the induction of apoptosis. In addition, CMC induced activation of caspase-3, and subsequent cleavage of PARP. The caspase signals may originate from mitochondrial dysfunction, which was supported by the findings of decreased mitochondria transmembrane potential and the lower oxygen consumption rate. These results suggested that CMC has the potential to improve the treatment of chronic myeloid leukemia.

---

## **Renal Toxicity Studies of *Clematis terniflora* DC. var. *mandshurica* on rats**

Yuji Liang, Zhili Hu, Jie Li, Yan Wang, Rui Zheng

*Department of Pharmacology, Institute of Materia Media, Shandong Academy of Medical Sciences, Key Laboratory for Rare & Uncommon Diseases of Shandong Province, Ji'nan, China.*

**Objectives:** To investigate the renal toxicity of *Clematis terniflora* DC.var.*mandshurica* on rats and its possible mechanism.

**Methods:** 80 rats (male: female=1:1) were divided into four groups according to weight, and given *Clematis terniflora* DC.var.*mandshurica* extract (5.0, 12.5 and 25.0g/kg) or distilled water for 12 weeks, and observed 4 weeks after withdrawing the administration. Renal function including BUN, CRE, LDH, GGT and ALP was investigated by measuring the blood and urine on the 4th, 8th, 12th week during dosing period and on the 4th week after withdrawing dosing. At the same time the renal pathology were observed in part of rats.

**Results:** Urinary LDH in high dose group was significantly higher than that in control group on 4<sup>th</sup> dosing week. Urinary LDH and GGT in medium and high dose groups were significantly higher than that in control group, as well as pathology changes including focal renal tubular epithelial cell vacuolar degeneration on the junction of renal cortex and medulla were abseved on the 8<sup>th</sup> dosing week. Much heavier pathology changes such as diffuse renal tubular epithelial cell on the junction of renal cortex and medulla appeared hydropic morbid change of different degree in low, medium and high dose groups, protein casts dyed by eosin were visualized in most lumens, were detected on the 12<sup>th</sup> dosing week,

**Conclusions:** *Clematis terniflora* DC.var.*mandshurica* could induce significant renal toxicities when it was given long time in rats, which might be cause the damage in renal tubular epithelium cells.

**Keywords:** *Clematis terniflora* DC.var. *mandshurica*, renal toxicity, rat

## **Tumor growth suppression that makes GEF-1/Hgs a molecular target**

Kiyoshi Ogura, Yuko Niino, Reiko Minamikawa-Tachino

*Tokyo Metropolitan Institute of Medical Science, Laboratory of Tumor Therapy.*

GalCer expression factor-1 (GEF-1) /Hgs is an endosome transport-related protein and involved in TGF-beta-SMAD signal transduction. We found that GEF-1 induced Epithelial-Mesenchymal Transition (EMT). EMT is a fundamental process in the early stages of embryo morphogenesis induced by a signal like Wnt. EMT is also related to cancer metastasis. In order to clarify the role to the cancer metastasis of GEF-1, we generated murine malignant melanoma B16 mutant cell line (B16/GEF-1 cell) that overexpressed GEF-1. Overexpression of the GEF-1 in the B16/GEF-1 cells increased TGF-beta-SMAD signal transduction, and promoted cancer cell properties, such as cancer cell metastatic ability, angiogenic ability and tumor growth ability. GEF-1 was considered to be related to the cancer cell properties through TGF-beta-SMAD signal transduction. Therefore we established two B16 mutant cell lines which make GEF-1 a molecular target. B16/shRNA cells that overexpressed shRNA for GEF-1 were decreased the expression of GEF-1. B16/C cells overexpressed a c-domain of the GEF-1, which was a competitor for GEF-1 in TGF-beta-SMAD signal transduction. The cancer cell properties were suppressed in the B16/shRNA cells and the B16/C cells strongly. GEF-1/Hgs is expected as a molecular target for anti-tumor drug discovery.



## Effects of domain shuffling in VH-CDR3 between MLS128 and 83D4 on their Tn-antigen specificities

Hajime Sato<sup>1</sup>, Atsufumi Nakazawa<sup>1</sup>, Yoshihiro Kitabora<sup>1</sup>, Misato Maeda<sup>1</sup>, Akiho Shimura<sup>1</sup>, Noriyuki Yuasa<sup>1</sup>, Yoshiki Yamaguchi<sup>2</sup>, Yoko Fujita-Yamaguchi<sup>1</sup>

1. Department of Applied Biochemistry, Tokai University School of Engineering, Hiratsuka, Kanagawa, 259-1292, Japan;
2. Structural Glycobiology Team, Systems Glycobiology Research Group, RIKEN, Wako, Saitama 351-0198, Japan.

**Introduction:** Oncogenic transformation is often associated with dysregulation of glycosylation processes that then leads to altered patterns of carbohydrate functionalization on the surface of cancer cells. Tn-antigen (GalNAc $\alpha$ -Ser/Thr) and T-antigen (Gal $\beta$ 1-3GalNAc $\alpha$ -Ser/Thr) are antigens associated with carcinomas and are generally masked by covalently linked terminal carbohydrate moieties in normal human tissues but are exposed in most primary and metastatic epithelial malignant tumors. MLS128 and 83D4 are Tn-antigen specific monoclonal antibodies which belong to IgG and IgM, respectively. They bind carbohydrate epitopes consisting of three or two consecutive Tn-antigens (Tn3 or Tn2)<sup>1-4</sup>. Since MLS128 binds to a synthetic Tn3-peptide with approximately 10 times the affinity that it binds to a synthetic Tn2 peptide<sup>3</sup>, and since VH sequences of both mAbs are nearly identical except a few amino acids in the CDR3<sup>5</sup>, the aim of this study was to find out whether or not the VH CDR3 play a role in determining their Tn-antigen specificities.

**Methods:** The VH Domain of 83D4 was cloned into pCI-MLS128 VL-Fc to construct pCI/83D4-MLS128 vector. CHO cells were transfected with the vector. After 83D4-MLS128 scFv-Fc expression was confirmed, CHO cells were cultured in a selective medium to screen clones expressing high levels of 83D4-MLS128 scFv-Fc. From culture media of one stable clone, 83D4-MLS128 scFv-Fc proteins were purified by Protein A-Sepharose chromatography to characterize Tn-antigen specificity and affinity.

**Results and Conclusions:** After CHO cells were transiently transfected with the vector, 83D4-MLS128 scFv-Fc production was confirmed by detection of Fc in the medium using a Sandwich ELISA. Transfected cells were then cultured in the medium containing G418 for 2 weeks. Stable clones were screened by determining Fc-levels in their media. Of several stable clones producing the 83D4-MLS128 scFv-Fc protein at 1-2  $\mu$ g/ml levels established, one clone was chosen for purification and characterization of the expressed 83D4-MLS128 scFv-Fc protein. From 80 ml of culture medium, 55 $\mu$ g of 83D4-MLS128 scFv-Fc protein were purified to near homogeneity by Protein A-Sepharose chromatography. Further studies including complete purification and Tn-antigen specificity and affinity determination are in progress.

### References:

1. Numata Y *et al.* A monoclonal antibody directed to Tn antigen. *Biochem Biophys Res Commun* 1990; 170:981-5.
2. Nakada H *et al.* Epitopic structure of Tn glycoprotein A for an anti-Tn antibody (MLS128). *Proc Natl Acad Sci USA*. 1993; 90:2495-9.
3. Osinaga E *et al.* Analysis of the fine specificity of Tn-binding proteins using synthetic glycopeptide epitopes and a biosensor based on surface plasmon resonance spectroscopy. *FEBS Lett*. 2000; 469:24-8.
4. Matsumoto-Takasaki A *et al.* Surface plasmon resonance and NMR analyses of anti Tn-antigen MLS128 monoclonal antibody binding to two or three consecutive Tn-antigen clusters. *J Biochem*. 2012; 151:273-82.
5. Yuasa N *et al.* Production of anti-carbohydrate antibodies by phage-display technologies: Potential impairment of cell growth as a result of endogenous expression. *J Biochem*. 2012; 151:371-81.

## Cancer cell migration and distribution in reconstructed basal membrane-stromal tissues with collagen gels

Yo Kamoshida<sup>1</sup>, Mariko Muto<sup>1</sup>, Shuya Mori<sup>1</sup>, Mioto Yoshida<sup>1</sup>, Keita Yoshimura<sup>2</sup>, Taisuke Kurokawa<sup>1</sup>, Chihiro Fukayama<sup>1</sup>, Kyohei Fukuda<sup>1</sup>, Wei Tang<sup>3</sup>, Yoko Fujita-Yamaguchi<sup>1</sup>, Munehiro Nakata<sup>1</sup>

1. Department of Applied Biochemistry, Tokai University, Hiratsuka, Kanagawa, Japan;

2. Educational Support Center, Tokai University, Hiratsuka, Kanagawa, Japan;

3. Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

**Objectives:** Cancer cells that arise in epithelial tissues destroy basement membrane and invade into stromal tissues. Matrix metalloproteinases (MMPs) are involved in the invasion process which include degradation of extracellular matrix components such as type IV collagen in basement membrane and type I collagen in stromal tissues. Since cancer cell invasion is the initial step of metastasis, understanding this invasion process would greatly advance cancer research towards controlling metastasis of cancer cells. The present study was aimed to establish an observation method for cancer cell invasion into model basal membrane-stromal tissues reconstituted with type IV and I collagens, respectively. We prepared the reconstituted type I and IV collagen gels to examine migration of human breast carcinoma MDA-MB-231 cells into the gels. Microscopic observation was carried out using the frozen sections of the collagen gels to which MDA-MB-231 cells invaded in the absence or presence of potential inhibitors.

**Methods:** Human breast carcinoma cell line MDA-MB-231 was maintained in DMEM containing 5% fetal calf serum (FCS). Type I collagen was commercially obtained. Type IV collagen from porcine lens capsules was purified by acid extraction followed by anion-exchange chromatography. Type I and type IV collagen solutions were allowed to form the gels in chamber slide wells. The gels on which the cells were incubated for 18 h were subjected to freeze-sectioning followed by azan staining and microscopic observation.

**Results and Discussion:** Histochemical observation of MDA-MB-231 cells in type I collagen gels showed that some cells remained on the gel surface and the others migrated into the gel with a wide range of distribution from the gel surface, suggesting that each cell possesses individuality for migration ability. The cell migration was promoted in the presence of FCS whereas it was suppressed in the presence of galardin known as an MMP inhibitor. The inhibition of cell migration by galardin was also observed when the cells were incubated on type IV collagen gels. Effect of cinobufacini, an extract of *Bufo bufo* gargarizans skin, of which apoptosis induction ability against cancer cells has been revealed, was also examined on cell migration into type I collagen gels. Cinobufatini showed not only some toxicity against MDA-MB-231 cells but also inhibitory effect on the migration of the survived cells in a dose-dependent manner. These results suggest that a combination of reconstituted collagen gels and microscopic observation of cancer cells in the freeze-section is a useful method to investigate cancer cell migration and invasion and that this method would be applicable to evaluate invasion inhibitory effect of pharmaceuticals of interest.

---

## **Methylamine irisolidone, a novel compound, prevents hypoxia-induced apoptosis through maintaining mitochondrial membrane potential in cardiomyocytes**

Yanling Mu, Fengling Wang, Ling Zhou, Ju Wang, Yanying Xie

*Shandong Academy of Medical Sciences, Institute of Materia Medica, Key Laboratory for Rare Disease Research of Shandong Province, Ji'nan, Shandong, China.*

Methylamine irisolidone (8-methylenemethylamine-irisolidone, MMI) is a structural modified novel compound from kakkalide, which is isolated from *Flos Puerariae*. Given that MMI can protect heart against ischemia as described as our previous studies, we investigated, in this study, its inhibiting effect of apoptosis in cardiomyocytes (H9c2 cell line) and the underlying mechanism during hypoxia. The results demonstrated that MMI increased cell viability in a concentration-dependent manner in H9c2 cells during hypoxia. Application of MMI also prevents hypoxia induced apoptosis in H9c2 cells. Further study indicates that MMI can prevent the depolarization of mitochondrial membrane potential during hypoxia and promotes the activity of ATPase in H9c2 cells. The study demonstrates that MMI can prevent hypoxia induced apoptosis and enhance cell viability of H9c2 cells. The protective effects of MMI may be associated with its ability to prevent mitochondrial membrane depolarization and promote the total ATPase activity during hypoxia.

**Keywords:** methylamine irisolidone, myocardial ischemia, mitochondrial membrane potential, apoptosis, ATPase



## Guide for Authors

### 1. Scope of Articles

Drug Discoveries & Therapeutics welcomes contributions in all fields of pharmaceutical and therapeutic research such as medicinal chemistry, pharmacology, pharmaceutical analysis, pharmaceuticals, pharmaceutical administration, and experimental and clinical studies of effects, mechanisms, or uses of various treatments. Studies in drug-related fields such as biology, biochemistry, physiology, microbiology, and immunology are also within the scope of this journal.

### 2. Submission Types

**Original Articles** should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables.

**Brief Reports** definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

**Reviews** should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of 100 references. Mini reviews are also accepted.

**Policy Forum** articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 2,000 words in length (excluding references).

**Case Reports** should be detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient but usually describe an unusual or novel occurrence. Unreported or unusual side effects or adverse interactions involving medications will also be considered. Case

Reports should not exceed 3,000 words in length (excluding references).

**News** articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

**Letters** should present considered opinions in response to articles published in Drug Discoveries & Therapeutics in the last 6 months or issues of general interest. Letters should not exceed 800 words in length and may contain a maximum of 10 references.

### 3. Editorial Policies

**Ethics:** Drug Discoveries & Therapeutics requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board.

**Conflict of Interest:** All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

**Submission Declaration:** When a manuscript is considered for submission to Drug Discoveries & Therapeutics, the authors should confirm that 1) no part of this manuscript is currently under consideration for publication elsewhere; 2) this manuscript does not contain the same information in whole or in part as manuscripts that have been published, accepted, or are under review elsewhere, except in the form of an abstract, a letter to the editor, or part of a published lecture or academic thesis; 3) authorization for publication has been obtained from the authors' employer or institution; and 4) all contributing authors have agreed to submit this manuscript.

**Cover Letter:** The manuscript must be accompanied by a cover letter signed by the corresponding author on behalf of all authors. The letter should indicate the basic findings of the work and their significance. The letter should also include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been published previously or is not under consideration for publication elsewhere. The cover letter should be submitted in PDF format. For example of Cover Letter, please visit <http://www.ddtjournal.com/downloadcentre.php> (Download Centre).

**Copyright:** A signed JOURNAL PUBLISHING AGREEMENT (JPA) must be provided by post, fax, or as a scanned file before acceptance of the article. Only forms with a hand-written signature are accepted. This copyright will ensure the widest possible dissemination of information. A form facilitating transfer of copyright can be downloaded by clicking the appropriate link and can be returned to the e-mail address or fax number noted on the form (Please visit

Download Centre). Please note that your manuscript will not proceed to the next step in publication until the JPA form is received. In addition, if excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

**Suggested Reviewers:** A list of up to 3 reviewers who are qualified to assess the scientific merit of the study is welcomed. Reviewer information including names, affiliations, addresses, and e-mail should be provided at the same time the manuscript is submitted online. Please do not suggest reviewers with known conflicts of interest, including participants or anyone with a stake in the proposed research; anyone from the same institution; former students, advisors, or research collaborators (within the last three years); or close personal contacts. Please note that the Editor-in-Chief may accept one or more of the proposed reviewers or may request a review by other qualified persons.

**Language Editing:** Manuscripts prepared by authors whose native language is not English should have their work proofread by a native English speaker before submission. If not, this might delay the publication of your manuscript in Drug Discoveries & Therapeutics.

The Editing Support Organization can provide English proofreading, Japanese-English translation, and Chinese-English translation services to authors who want to publish in Drug Discoveries & Therapeutics and need assistance before submitting a manuscript. Authors can visit this organization directly at <http://www.iacmhr.com/iac-eso/support.php?lang=en>. IAC-ESO was established to facilitate manuscript preparation by researchers whose native language is not English and to help edit works intended for international academic journals.

### 4. Manuscript Preparation

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (e.g. DNA). Single words should not be abbreviated.

**Title page:** The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s); 3) abbreviated names of the author(s); 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose"). Please visit [Download Centre](#) and refer to the title page of the manuscript sample.

**Abstract:** A one-paragraph abstract consisting of no more than 250 words must be included. The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Key words or phrases that do not occur in the title should be included in the Abstract page.

**Introduction:** The introduction should be a concise statement of the basis for the study and its scientific context.

**Materials and Methods:** The description should be brief but with sufficient detail to enable others to reproduce the experiments. Procedures that have been published previously should not be described in detail but appropriate references should simply be cited. Only new and significant modifications of previously published procedures require complete description. Names of products and manufacturers with their locations (city and state/country) should be given and sources of animals and cell lines should always be indicated. All clinical investigations must have been conducted in accordance with Declaration of Helsinki principles. All human and animal studies must have been approved by the appropriate institutional review board(s) and a specific declaration of approval must be made within this section.

**Results:** The description of the experimental results should be succinct but in sufficient detail to allow the experiments to be analyzed and interpreted by an independent reader. If necessary, subheadings may be used for an orderly presentation. All figures and tables must be referred to in the text.

**Discussion:** The data should be interpreted concisely without repeating material already presented in the Results section. Speculation is permissible, but it must be well-founded, and discussion of the wider implications of the findings is encouraged. Conclusions derived from the study should be included in this section.

**Acknowledgments:** All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not meet the criteria for authors should be listed along with their contributions.

**References:** References should be numbered in the order in which they appear in the text. Citing of unpublished results, personal communications, conference abstracts, and theses in the reference list is not recommended but these sources may be mentioned in the text. In the reference list, cite the names of all authors when there are fifteen or fewer authors; if there are sixteen or more authors, list the first three followed by *et al.* Names of journals should be abbreviated in the style used in PubMed. Authors are responsible for the accuracy of the references. Examples are given below:

*Example 1 (Sample journal reference):*  
Nakata M, Tang W. Japan-China Joint Medical Workshop on Drug Discoveries and Therapeutics 2008: The need of Asian pharmaceutical researchers' cooperation. *Drug Discov Ther.* 2008; 2:262-263.

*Example 2 (Sample journal reference with more than 15 authors):*  
Darby S, Hill D, Auvinen A, *et al.* Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. *BMJ.* 2005; 330:223.

*Example 3 (Sample book reference):*  
Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: *Post-traumatic Stress Disorder, Diagnosis, Management and Treatment* (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

*Example 4 (Sample web page reference):*  
World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. [http://www.who.int/whr/2008/whr08\\_en.pdf](http://www.who.int/whr/2008/whr08_en.pdf) (accessed September 23, 2010).

**Tables:** All tables should be prepared in Microsoft Word or Excel and should be arranged at the end of the manuscript after the References section. Please note that tables should not in image format. All tables should have a concise title and should be numbered consecutively with Arabic numerals. If necessary, additional information should be given below the table.

**Figure Legend:** The figure legend should be typed on a separate page of the main manuscript and should include a short title and explanation. The legend should be concise but comprehensive and should be understood without referring to the text. Symbols used in figures must be explained.

**Figure Preparation:** All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column, 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Please make sure that artwork files are in an acceptable format (TIFF or JPEG) at minimum resolution (600 dpi for illustrations, graphs, and annotated artwork, and 300 dpi for micrographs and photographs). Please provide all figures as separate files. Please note that low-resolution images are one of the leading causes of article resubmission and schedule delays. All color figures will be reproduced in full color in the online edition of the journal at no cost to authors.

**Units and Symbols:** Units and symbols conforming to the International System of Units (SI) should be used for physicochemical quantities. Solidus notation (*e.g.* mg/kg, mg/mL, mol/mm<sup>2</sup>/min) should be used. Please refer to the SI Guide [www.bipm.org/en/si/](http://www.bipm.org/en/si/) for standard units.

**Supplemental data:** Supplemental data might be useful for supporting and enhancing your scientific research and

Drug Discoveries & Therapeutics accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g.*, Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

## 5. Submission Checklist

The Submission Checklist will be useful during the final checking of a manuscript prior to sending it to Drug Discoveries & Therapeutics for review. Please visit [Download Centre](#) and download the Submission Checklist file.

## 6. Online submission

Manuscripts should be submitted to Drug Discoveries & Therapeutics online at <http://www.ddtjournal.com>. The manuscript file should be smaller than 5 MB in size. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail at [office@ddtjournal.com](mailto:office@ddtjournal.com)

## 7. Accepted manuscripts

**Proofs:** Galley proofs in PDF format will be sent to the corresponding author *via* e-mail. Corrections must be returned to the editor ([proof-editing@ddtjournal.com](mailto:proof-editing@ddtjournal.com)) within 3 working days.

**Offprints:** Authors will be provided with electronic offprints of their article. Paper offprints can be ordered at prices quoted on the order form that accompanies the proofs.

**Page Charge:** A page charge of \$140 will be assessed for each printed page of an accepted manuscript. The charge for printing color figures is \$340 for each page. Under exceptional circumstances, the author(s) may apply to the editorial office for a waiver of the publication charges at the time of submission.

(Revised October 2011)

## Editorial and Head Office:

Pearl City Koishikawa 603  
2-4-5 Kasuga, Bunkyo-ku  
Tokyo 112-0003  
Japan  
Tel: +81-3-5840-9697  
Fax: +81-3-5840-9698  
E-mail: [office@ddtjournal.com](mailto:office@ddtjournal.com)

## JOURNAL PUBLISHING AGREEMENT (JPA)

-----  
**Manuscript No.:**

**Title:**

**Corresponding author:**  
-----

The International Advancement Center for Medicine & Health Research Co., Ltd. (IACMHR Co., Ltd.) is pleased to accept the above article for publication in Drug Discoveries & Therapeutics. The International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) reserves all rights to the published article. Your written acceptance of this JOURNAL PUBLISHING AGREEMENT is required before the article can be published. Please read this form carefully and sign it if you agree to its terms. The signed JOURNAL PUBLISHING AGREEMENT should be sent to the Drug Discoveries & Therapeutics office (Pearl City Koishikawa 603, 2-4-5 Kasuga, Bunkyo-ku, Tokyo 112-0003, Japan; E-mail: office@ddtjournal.com; Tel: +81-3-5840-9697; Fax: +81-3-5840-9698).

### 1. Authorship Criteria

As the corresponding author, I certify on behalf of all of the authors that:

- 1) The article is an original work and does not involve fraud, fabrication, or plagiarism.
- 2) The article has not been published previously and is not currently under consideration for publication elsewhere. If accepted by Drug Discoveries & Therapeutics, the article will not be submitted for publication to any other journal.
- 3) The article contains no libelous or other unlawful statements and does not contain any materials that infringes upon individual privacy or proprietary rights or any statutory copyright.
- 4) I have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in my article.
- 5) All authors have made significant contributions to the study including the conception and design of this work, the analysis of the data, and the writing of the manuscript.
- 6) All authors have reviewed this manuscript and take responsibility for its content and approve its publication.
- 7) I have informed all of the authors of the terms of this publishing agreement and I am signing on their behalf as their agent.

### 2. Copyright Transfer Agreement

I hereby assign and transfer to IACMHR Co., Ltd. all exclusive rights of copyright ownership to the above work in the journal Drug Discoveries & Therapeutics, including but not limited to the right 1) to publish, republish, derivate, distribute, transmit, sell, and otherwise use the work and other related material worldwide, in whole or in part, in all languages, in electronic, printed, or any other forms of media now known or hereafter developed and the right 2) to authorize or license third parties to do any of the above.

I understand that these exclusive rights will become the property of IACMHR Co., Ltd., from the date the article is accepted for publication in the journal Drug Discoveries & Therapeutics. I also understand that IACMHR Co., Ltd. as a copyright owner has sole authority to license and permit reproductions of the article.

I understand that except for copyright, other proprietary rights related to the Work (e.g. patent or other rights to any process or procedure) shall be retained by the authors. To reproduce any text, figures, tables, or illustrations from this Work in future works of their own, the authors must obtain written permission from IACMHR Co., Ltd.; such permission cannot be unreasonably withheld by IACMHR Co., Ltd.

### 3. Conflict of Interest Disclosure

I confirm that all funding sources supporting the work and all institutions or people who contributed to the work but who do not meet the criteria for authors are acknowledged. I also confirm that all commercial affiliations, stock ownership, equity interests, or patent-licensing arrangements that could be considered to pose a financial conflict of interest in connection with the article have been disclosed.

-----  
**Corresponding Author's Name (Signature):**

**Date:**

