# 6(ii): Targeting anti cancer drug

**Session Chair**
Dr. Takahiro Ochiya  
National Cancer Center Research Institute, Japan

**Session Co-Chair**
Dr. Chulso Moon  
Johns Hopkins University School of Medicine, USA

## Session Introduction

<table>
<thead>
<tr>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cancer-relevant functions of the plasma membrane receptor for thyroid hormone analogues</strong></td>
<td>Dr. Paul J. Davis, Albany Medical College, USA</td>
</tr>
<tr>
<td><strong>Ribophorin II (RPN2) as a novel therapeutic target for cancer stem cells</strong></td>
<td>Dr. Takahiro Ochiya, National Cancer Center Research Institute, Japan</td>
</tr>
<tr>
<td><strong>Aquaporin 5 (AQP5) activates the epidermal growth factor receptor (EGFR) and Src, potentially through its novel kinase activity, and may be involved in Iressa resistance</strong></td>
<td>Dr. Chulso Moon, Johns Hopkins University School of Medicine, USA</td>
</tr>
<tr>
<td><strong>Analysis of miR-195 and miR-497 expression, regulation and role in breast cancer</strong></td>
<td>Dr. Lihui Lai, East China Normal University, China</td>
</tr>
<tr>
<td><strong>Cucurbitacin B enhances the cytotoxicity of doxorubicin by increasing intracellular drug accumulation</strong></td>
<td>Dr. Meixia Zhang, China Medical University, China</td>
</tr>
<tr>
<td><strong>Multifunctional nanoparticles for targeted drug delivery and MRI contrast agent</strong></td>
<td>Dr. Panchanan Pramanik, Indian Institute of Technology-Kharagpur, India</td>
</tr>
</tbody>
</table>
Cancer-Relevant Functions of the Plasma Membrane Receptor for Thyroid Hormone Analogues

Paul J. Davis, Hung-Yun Lin, Vivian Cody, Faith B. Davis, Aleck Hercbergs and Shaker A. Mousa
Ordway Research Institute and Albany College of Pharmacy and Health Sciences, USA

Integrin αvβ3 is a heterodimeric structural protein of the plasma membrane that contains a high affinity receptor for thyroid hormone. Functions of this receptor are wholly distinct from those of the classical nuclear receptor (TR) for thyroid hormone. The hormone receptor on αvβ3 enables L-thyroxine (T4) and 3, 5, 3’-triiodo-L-thyronine (T3) to stimulate cancer cell proliferation and angiogenesis. A deaminated derivative of T4, tetraiodothyroacetic acid (tetrac), blocks binding and proliferative actions of T4 and T3 at the αvβ3 receptor; tetrac also has anti-proliferative actions at the thyroid hormone receptor in the absence of T4 and T3. Structure-activity relationships of hormone analogues at the receptor have been computer-modeled and indicate the receptor includes a site (S1) that binds T3 and a site (S2) for which both T4 and T3 are ligands. Tetrac acts at both S1 and S2. Cell proliferation is modulated from the S2 site. Tetrac has been re-formulated as a nanoparticle (nanotetrac) that acts exclusively at the αvβ3 receptor and does not enter cells. Nanotetrac 1) disorders expression of genes in multiple cancer cell survival pathways 2) blocks human cancer cell proliferation in vitro and in tumor xenografts and 3) inhibits the pro-angiogenic actions in vitro of VEGF, bFGF and other growth factors. Nanotetrac radiosensitizes cancer cells by inhibiting repair of double-strand DNA breaks. Thus, the receptor described on integrin αvβ3 for T4 and T3 provides insight into tumor cell and vascular cell biology and tetrac formulations offer a novel, disabling effects on multiple cancer cell defense pathways.

Biography

Paul J. Davis obtained his M.D. at Harvard Medical School and his clinical training at Albert Einstein College of Medicine and the NIH. He is Senior Associate Dean for Clinical Research at Albany Medical College and a senior faculty at Albany College of Pharmacy and Health Sciences. He was a founder of Ordway Research Institute, a nonprofit translational biomedical research company. He is a basic science endocrinologist, is co-author of more than 200 original papers and 25 textbook chapters and is an Associate Editor of two medical journals.
Ribophorin II (RPN2) as a novel therapeutic target for cancer stem cells

Takahiro Ochiya
National Cancer Center Research Institute, Japan

The survival rate for women with advanced, metastatic breast cancer has not changed significantly for decades. Regardless of effective therapies, many women still experience recurrences of breast cancer after treatment. Docetaxel has been shown to be beneficial in the treatment of breast cancer; however, almost half of treated patients do not respond to it and many tumors develop resistance. At present no method exists to predict response to docetaxel or to detect resistance. Moreover, target molecules to increase the efficacy of chemotherapy have not yet been identified. Here we found that inhibition of the ribophorin II (RPN2), a part of oligosaccharyltransferase (OST) complex, promoted docetaxel-dependent apoptosis and inhibited cell growth in a docetaxel-resistant human breast cancer cell line. Silencing of RPN2 resulted in decreased glycosylation and membrane localization of the P-glycoprotein efflux pump, which caused increased sensitization of drug resistant cells to docetaxel (Nat Med, 2008). We also currently found that RPN2 is highly expressed in breast cancer stem cells. Knockdown of RPN2 in cancer stem cells by shRPN2 vector system allowed a significant inhibition of cancer growth and lymph node metastasis in vivo. We also found that small non-coding RNA tightly regulates RPN2 gene expression. RPN2 could, therefore, have clinical applications as a target for micromanaging cancer stem cells.

Biography
Dr. Takahiro Ochiya is Chief of Division of Molecular and Cellular Medicine at the National Cancer Center Research Institute, Tokyo and he is also appointed as a invited professor of Waseda University (since 2004) and Tokyo Institute of Technology (since 2008). After he got Ph.D. in 1988 in Osaka University and then went to do a post-doc at La Jolla Cancer Research (Burnham Institute for Medical Research), CA, USA. Dr. Ochiya's lab focuses the development of novel animal models, methods, and strategies to study cancer development and metastasis. Especially, current focus are siRNA- and microRNA-based therapy against cancer stem cells.
Aquaporin 5 (AQP5) activates the epidermal growth factor receptor (EGFR) and Src, potentially through its novel kinase activity, and may be involved in Iressa resistance

Chulso Moon
Johns Hopkins University School of Medicine, USA

The role of aquaporin water channels in human carcinogenesis (AQPs) recently has become an area of great interest. We have previously demonstrated that AQP5 can promote cell proliferation leading to tumorigenesis by activation ERK1/2 pathways and that the expression of AQP5 is associated with prognosis of lung cancer, colon cancer and CML. Here, we provide evidences that these phenomenon may be mediated by activation of EGFR and/or Src, upstream signal for ERK1/2. Cellular hyperplasia and activation of ERK1/2 in transgenic mice carrying human AQP5 over-expression construct confirms our prior findings in vivo. Expression of AQP5 activate EGFR and Src in BEAS cells and HCT116 cells by increasing phosphorylation of both EGFR and Src, and inhibition of AQP5 expression lead to decreased phosphorylation of both molecules with decreased ERK1/2 activation. The association of AQP5 with EGFR and Src are demonstrated by immnoprecipitation and immnoflourescence examinations in BEAS cells and such interaction is inhibited by mutation in PKA site in AQP5. Furthermore, by using baculovirus system, recombinant hAQP5 (rAQP5) was purified, which shows a unique kinase activity in vitro and that rAQP5 can synergistically increase overall kinase activity when combined with EGFR and Src. Based on these findings, we demonstrate that AQP5 may be involved in the development of Iressa (a small molecular inhibitor for EGFR) resistances, possibly through modulating phosphorylation of EGFR. While these observations provide several novel findings, studies for the detailed mechanistics leading to activation of EGFR and Src by AQP5 are warranted in the future.

Biography

Dr. Chulso Moon MD, PhD is a board certified medical oncologist in US and has been working in Johns Hopkins University (JHU) since 2001 as tenure track faculty, attending physician in the department of otolaryngology/oncology and JHU Cancer Center. Presently, he is actively participating in the cancer research as adjunct professorship in JHU and also mentoring graduate student in human genetics program in Johns Hopkins Medical School. He is an MD, PhD physician scientist participating both academic research and patient care. He obtained his PhD in human genetics from JHU under Dr. Peter Agre (2003 Novel Laureate) and finished his medicine and oncology training in MD Anderson Cancer Center. He played a key role in characterizing the role of AQPs in human cancer by providing the first model of AQPS as a novel therapeutic target. Additionally, he has published several key review articles in clinical oncology focused on head and neck and prostate cancer.
Analysis of miR-195 and miR-497 expression, regulation and role in breast cancer

Dan Li1, Yulan Zhao1, Changxing Liu1, Xiaona Chen1, Yanting Qi2, Yue Jiang1,3, Chao Zou1, Xiaolong Zhang1, Shunying Liu1, Xuejing Wang1, Chuan-Xiu Bian1, Dan Zhao1, Qiang Sun1, Zhenbing Zeng1, Andreas Dress2, Marie C. Lin1,6, Hsiang-Fu Kung1,5,1, Feng Mao4, Bing-Hua Jiang2,1 and Lihui Lai1

1Institute of Molecular and Chemical Biology, East China Normal University, Shanghai, China
2Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA
3Department of General Surgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China
4Department of Breast Surgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China
5Faculty of Medicine, The Chinese University of Hong Kong, HK, SAR, China
6Brain Tumor Center, Neurosurgery Division, Faculty of Medicine, PWH, The Chinese University of Hong Kong, Shatin, Hong Kong, China
7CAS-MPG Partner Institute and Key Lab for Computational Biology, SIBS, CAS, Shanghai, China

Purpose: To investigate expression, regulation, potential role and targets of miR-195 and miR-497 in breast cancer.

Experimental design: The expression patterns of miR-195 and miR-497 were initially examined in breast cancer tissues and cell lines by Deep sequencing: Northern blotting and quantitative real-time PCR. Combined bisulfite restriction analysis and bisulfite sequencing were carried out to study the DNA methylation status of miR-195/497 gene. Breast cancer cells stably expressing miR-195 or miR-497 were established to study their role and targets. Finally, normal, fibroadenoma and breast cancer tissues were employed to analyze the correlation between miR-195/497 levels and malignant stages of breast tumor samples.

Results: MiR-195 and miR-497 were significantly down-regulated in breast cancer. The methylation state of CpG islands upstream of the miR-195/497 gene was found to be responsible for the down-regulation of both miRNAs. Forced expression of miR-195 or miR-497 suppressed breast cancer cell proliferation and invasion. Raf-1 was identified as a novel direct target of miR-195 and miR-497. MiR-195/497 expression levels in clinical specimens were found to be correlated inversely with malignancy of breast cancer.

Conclusion: Our data imply that both miR-195 and miR-497 play important inhibitory roles in breast cancer malignancy and may be the potential therapeutic and diagnostic targets.
Cucurbitacin B enhances the cytotoxicity of doxorubicin by increasing intracellular drug accumulation

Meixia Zhang¹, Jiao Yang²,³, Xueying Zhou¹, Yanli Wang¹, Wen Li³, Jesse Li-Ling⁴ and Yihui Deng⁴

¹Department of Clinical Pharmacology, China Medical University, China
²Department of Pharmacy, The General Hospital of Daqing Oilfield, China
³Department of Medical Genetics, China Medical University, China
⁴Department of Pharmacy, Shenyang Pharmaceutical University, China

**Purpose:** To assess the effect of cucurbitacin B (CuB) on the cytotoxicity of doxorubicin (Dox) in human hepatocellular carcinoma cells and to explore the potential mechanisms.

**Methods:** The cytotoxicity of combined CuB and Dox in human hepatocellular carcinoma cell line (HepG2) was investigated with a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. The effect of CuB on Dox concentration in HepG2 cells was determined by evaluating the influx of Dox and the efflux of Dox from such cells. In vivo effect of combined CuB and Dox on the growth of murine H22 cells was also determined.

**Results:** Our data demonstrated that the cytotoxicity by CuB and Dox was additive in HepG2 cells. CuB has significantly increased Dox concentration in tumor cells by promoting Dox influx and suppressing Dox efflux. In vivo anti-tumor activity assay also showed that the combination of two drugs can result in more significant tumor regression compared with single drug usage.

**Conclusion:** Our results suggested that combined CuB and Dox may be an effective regime for the chemotherapy of HCC.
Multifunctional nanoparticles for targeted drug delivery and MRI contrast agent

P. Pramanik
Nanomaterials Laboratory, Indian Institute of Technology-Kharagpur, India

Superparamagnetic iron oxide nanoparticles offer a unique carrier system, whose surface can be modified with multiple diagnostic and therapeutic entities to serve as both targeting contrasts and drug carriers simultaneously, allowing for real time monitoring of response from tumor to drug treatment. An effective approach towards improving the targeting capability and drug release efficiency of magnetic nanoparticles is to conjugate the nanoparticles with low molecular targeting agent, such as folic acid and small bio-molecule those have strong affinities for target cells and high efficiency for internalization of nanoparticle. Recently we have developed a series of novel technique to synthesize highly stable folic acid conjugated magnetite (Fe$_3$O$_4$) nanoparticles for targeting cancer cells, using derivatives of phosphonic acid and chitosan as vehicle. 2, 2'-(ethylenedioxy)-bis-ethylamine, a non-polymeric hydrophilic linker has been used as surface-coupling agent. These iron-oxide folate nano-conjugates are non-cytotoxic and shows high site-specific intracellular uptake against folate receptors over expressed onto cancer cells.

In our constant endeavor to design nanoparticles for site-specific drug targeting, "smart" superparamagnetic nanodevice has been developed which combines magnetic targeting, fluorescent-imaging, receptor-specific targeted delivery and pH responsive drug release into one system. The device has been synthesized by covalently grafting the widely used targeting agent folic acid, chemotherapeutic anticancer drugs and fluorochrome rhodamine isothiocyanate onto the surface of superparamagnetic magnetite nanoparticles, functionalized with surface anchoring agent. The decorated magnetite nanoparticles serve as the core material to allow magnetically guided drug delivery and helps to enhance contrast due to T2-weighted magnetic resonance. Magentically activated cell-sorting and confocal microscopy clearly establish that cells with over expressed with human folate receptors internalize efficiently the drug modified with nanoparticles than normal cells.

Biography

Prof P.Pramanik completed M.Sc and Ph.D from Indian Institute of Technology (IIT), Kharagpur, India. He is professor since 1993 in IIT, Kharagpur. His research interest is material chemistry and nano-biotechnology. He has publishes about 200 papers in international journals. He is member of many advisory board of government of India. He is key member of task force for nano-biotechnology of government of India.