323 Poster

mTOR inhibition reverses acquired endocrine therapy resistance of breast cancer cells at the cell proliferation and gene expression levels

P. Cohen[1], I. Bieche[2], J. Vendrell[3], C. Keime[4], R. Lidereau[2], C. Dumontet[5], S. Ghayad[1]
[1]INSERM U590, Faculté de Pharmacie, Lyon, France; [2] INSERM U375, Laboratoire d'Oncogénétique, Saint Cloud, France; [3] INSERM U590, Faculte de Pharmacie de Lyon, Saint Cloud, France; [4] PRABI, Université de Lyon, Lyon, France; [5] INSERM U590, Faculté de Médecine Rockfeller, Lyon, France

Cross-resistance to molecules used in endocrine therapy is among the main challenges in the treatment of estrogen receptor alpha (ERalpha) positive breast cancer patients and utmost importance is attached to strategies of reversion. The resistant ERalpha-positive MCF-7-derived cells used in this study have acquired both cross-resistance to OH-Tam and to ICI182,780 and strong activation of the Akt/mTOR pathway. Cell proliferation tests in control cells demonstrated that rapamycin had no effect when used alone, but it enhanced cell sensitivity to endocrine therapy when combined to OH-Tam or to ICI182,780. In resistant cells, rapamycin used alone greatly inhibited cell proliferation and reversed resistance to endocrine therapy by blocking the agonist-like activity of OH-Tam on cell proliferation and bypassing ICI182,780 resistance. Pangenomic DNA array experiments demonstrated that the co-treatment of resistant cells with ICI182,780 and rapamycin allowed the restoration of 40% of the ICI182,780 gene expression signature. We demonstrated that the reversion of endocrine therapy resistance by rapamycin was associated with increased ERalpha expression and decreased phospho-ser167 ERalpha/total ERalpha ratio. Taken together, our data strongly support the importance of using mTor inhibitors in the clinical management of ER+ endocrine therapy-resistant breast tumors.

324 Poster

Effects of a selective cyclooxygenase-1 inhibitor in SKOV-3 ovarian carcinoma xenograft-bearing mice

W. Li[1]
[1]Nanjing Medical University of Hangzhou Hospital, Department of Gynecology, Hangzhou, China

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are effective in both cancer prevention and as adjuvant therapy in the treatment of established tumors. To evaluate the effect of a cyclooxygenase-1 (COX-1) inhibitor, SC-560, on the growth inhibition of s.c. human ovarian SKOV-3 carcinoma and on angiogenesis.

Materials and methods: Human ovarian SKOV-3 carcinoma cells xenograft-bearing mice were treated with SC-560, a COX-1-selective inhibitor, 6 mg/kg alone i.g. daily and i.p. injections of cisplatin 3 mg/kg every other day for 21 days. Prostaglandin E2 (PGE2) levels was determined by ELISA. Microvessel density (MVD) of ovarian carcinoma was determined with anti-CD34 as the label by immunohistochemistry. In addition, the expression of COX-1 at protein and mRNA levels in the control group was detected by immunohistochemistry and RT-PCR.

Results: SC-560 reduced the growth of tumors when SKOV-3 cells were xenografted in nude female mice. The inhibitory rates in SC-560 group and cisplatin group were 47.1 % and 51.7 % respectively, which is significant statistically compared with that of control group (all, $P<0.05$). In treatment groups, SC-560 significantly reduced intratumor PGE2 levels ($P<0.01$). MVD in SC-560 group were 35.73 ± 9.87, which are significant statistically compared with that of control group (74.33 ± 9.50) ($P<0.01$). COX-1, not COX-2, mRNA and protein levels are elevated in tumor tissues.

Conclusions: These findings may implicate COX-1 as a suitable target for the treatment of ovarian cancer and that antiangiogenic therapy can be used to inhibit ovarian cancer growth.

325 Poster

Targeting p53 tumor suppressor to induce apoptosis and cell cycle arrest in esophageal cancer cells by novel sugar-cholestanols compounds

A. Faried[1], L.S. Faried[2], H. Kato[1], T. Asao[1], H. Kuwano[1], S. Yazawa[3]
[1]Gunma University Graduate school of Medicine, Department of Surgery, Maebashi Gunma, Japan; [2] Gunma University Graduate school of Medicine, Department of Gynecology, Maebashi Gunma, Japan; [3] Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan

Introduction: Our previous observation showed that sugar-chols had anticancer effect against a series of mouse and human cancer cells (1, 2). In this study, we evaluated a novel sugar-chols as an anticancer agent and elucidated the molecular basis of these compounds to induce apoptosis by affecting p53 signaling pathways in esophageal cancer cells.

Materials and Methods: Sugar-chols consisting of GlcNAc derivatives were synthesized through attaching to b-chol as an aglycon at the reducing-end. Anticancer potential against esophageal squamous cell carcinoma (ESCC) cells were evaluated by MTT. Further, molecular based changes to induce apoptosis and other pathways were examined by Western blotting.

Results: When ESCC cells were treated with GlcNAcGalChol and GlcNAcChol at 20μM, these sugar-chols were found to be taken into a cell and was associated with the following molecular based changes: First, up-regulation of HAUSP that stabilize p53 by deubiquitination cell growth repression and apoptosis. Second, activation of p53 pathways (p53 at ser 46), including two p53 family members, p63 and p73, as a favor selective binding of p53 to apoptotic promoters. Third, activation of Pin1to fully activate p53 resulting in the induction of apoptosis. Along with these changes, sugar-chols induced both up-regulation of p14ARF, Chk2, GADD-45, and p21CIP1 and down-regulation of MDM2 and Cyclin-E in a time-dependent manner.

Conclusion: Sugar-chols have been demonstrated to induce apoptosis in ESCC cells. Although the mechanism of such an induction with sugar-chols is not fully elucidated, it must be involved in the induction of p53 pathways leading to an irreversible inhibition of cell growth and cell cycle most decisively by activating apoptosis. This novel feature of sugar-chols should have clinical application by manipulated p53 pathway and as a promising anticancer agent for prevention and treatment of malignant diseases, especially esophageal cancer, in the near future.

References:
1. Faried A., et al. Seikagaku 2006; A10098 (3P-A-412).
2. Faried A., et al. Cancer Science 2007; 98: 1358-67.

326 Poster

Amydolytic detection of hepsin activity for non-invasive prostate cancer diagnostics

M. Savvateeva[1], E. Vasilieva[2], E. Kuznetsova[2]
[1]Lomonosov Moscow State University, Chemistry Enzyme Laboratory, Moscow, Russian Federation; [2] Moscow Research Institute of Medical Ecology, Laboratory of Protein Chemistry, Moscow, Russian Federation

Background. Hepsin is a membrane serine protease expressed in several normal human tissues including the liver, kidney, prostate, and thyroid. Recently, hepsin has been identified as one of the most up-regulated genes in prostate cancer (PC). The hepsin up-regulation appears to correlate with the disease progression. Materials and methods. To determine the ability of using hepsin as prostate cancer marker in urinary test we measured its amydolytic activity. Urine specimens from patients with prostate cancer, nonmalignant and benign prostate hyperplasia and normal donors were collected immediately after DRE (digital rectal examination). We measured the hepsin activity with chromogenic substrate P1-P2-P3-R-pNA under 405 nm. Active form of recombinant hepsin protein was used as positive control. Results. Hepsin activity detected in PC patients was 368±98 nmol/h*mkg protein, 96±72 nmol/h*mkg protein in patients with nonmalignant and benign prostatic hyperplasia and 17±9 nmol/h*mkg protein in normal donors. There was reliable difference between PC patients, normal donors and nonmalignant and benign prostate hyperplasia, so this molecular assay has potential application for distribution of patients into low- and high-risk groups for surveillance versus repeat biopsy. Conclusions. Our results demonstrated that a screening test based on hepsin detection in the urine specimens of patients with suspected prostate malignancy may be a probable substitution to serum screening tests based on determination of prostate specific antigen because of its high sensitivity and specificity.

327 Poster

Bisintercalating threading agents as cytotoxic inhibitors of transcription

A. Khazaly[1], Z. He[2], B. Stewart[2], G. Edwards[1], L. Wakelin[2]
[1]University of New South Wales, School of Chemistry, Randwick, Australia; [2] University of New South Wales, Physiology and Pharmacology, Randwick, Australia

Wakelin and colleagues have developed a series of bisintercalating threading dimers of 9-aminoacridine-4-carboxamides which are designed to be cytotoxic as a result of the template inhibition of transcription (Wakelin et al, J. Med. Chem. 2003, 46: 5790-5802). They are intended to bisintercalate into DNA from the minor groove, sandwiching 2 base pairs, and thread their carboxamide sidechains through the helix, so as to make hydrogen bonding interactions with the O6 and N7 atoms of guanine in the major groove. By binding in this manner, the side chain-guanine interaction makes withdrawal of the intercalated chromophores difficult, thereby slowing dissociation and providing a long-lived block to the passage of RNA