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

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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.

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

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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.

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

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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.

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

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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.

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Bisintercalating threading agents as cytotoxic inhibitors of transcription

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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

polymerase. Experiments with DNA in vitro confirm the threading binding mode and slow complex dissociation kinetics, and measurements using DNA microarrays have shown these agents to be powerful inhibitors of mRNA synthesis at cytotoxic doses. These findings confirm the importance of linker rigidity in kinetically stabilizing the DNA-ligand complex, and the importance of linker rigidity and slow kinetics in conferring template inhibition of transcription. Currently, we are exploring ways of enhancing these properties within the bis(9-aminoacridine-4-carboxamide) paradigm by structural modifications to the chromophore, the threading side-chain, and the linker itself. In the work described here, we report the synthesis and biological activity of a series of compounds in which a benzene ring has been fused to the acridine chromophore at the 5,6 position. In previous studies with monomeric 9-aminoacridine-4-carboxamides, this substitution has been shown to enhance DNA affinity, and to slow complex dissociation rates: effects attributed to enhanced stacking interactions between the intercalated chromophore and the DNA base pairs. In the dimer series, we find the benzacridine substitution enhances both cytotoxic potency and the life-time of the DNA complex.

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Cytotoxic activity of 4'-hydroxychalcone derivatives against Jurkat cells and their effects on mammalian DNA topoisomerase I

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Chalcones (1,3-diaryl-2-propen-1-ones) are alfa, beta-unsaturated ketones with cytotoxic and anticancer properties. Several reports have shown that the compounds with cytotoxic properties may also interfere with DNA topoisomerase functions. We synthesized five derivatives of 4'-hydroxychalcones and carried out cytotoxicity tests against transformed human T (Jurkat) cells as well as plasmid supercoil relaxation experiments using mammalian DNA topoisomerase I. The compounds synthesized was 3-aryl-1-(4'-hydroxyphenyl)-2-propen-1-one. The aryl part was phenyl, p-methylphenyl, p-methoxyphenyl, p-chlorophenyl and 2- thienyl for the compounds I-V respectively. The order of the cytotoxicity of the compounds was; IV > III > II > I > V. The compound IV, 3-(4-chlorophenyl)-1-(4'-hydroxyphenyl)-2-propen-1-one, had the highest Hammett and log P values (0.23 and 4.21, respectively) and exerted both highest cytotoxicity and strongest DNA topoisomerase I inhibition. The compounds I and II gave moderate interference with the DNA topoisomerase I while the remaining ones did not interfere with the enzyme.

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Biochemical and cellular effects of a novel cyclin-dependent kinase inhibitor

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Cyclin-dependent kinases (CDK) are essential components of the cell-cycle regulatory system and due to their frequent deregulations in cancer cells they have become important targets for drug development. We have recently prepared novel group of potent and selective CDK inhibitors based on pyrazolo[4,3-d]pyrimidine scaffold. The prototype derivative LGR1404 is an isomer of roscovitine, which is a well known CDK inhibitor. We therefore directly compared effects of both compounds in biochemical and cellular assays. As expected, compound LGR1404 was found to potently inhibit cyclin-dependent kinases CDK2, CDK5 and CDK9 in enzyme assays, with IC50 values in submicromolar range. Being more potent CDK inhibitor than roscovitine, the compound also demonstrated much stronger anti-proliferative activities in human cancer cell lines, including standardized NCI60 panel. An average GI50 for roscovitine is 19,3 μM, while LGR1404 has GI50 about 7 μM. Cells treated with LGR1404 show a dose-dependent decrease of phosphorylation of the retinoblastoma protein and cell cycle arrest. Moreover, the compound increases cellular level of the tumor suppressor protein p53, stabilizes its nuclear localization and, subsequently, activates transcription of some p53-regulated genes; this effect probably results from inhibition of CDKs involved in transcription. Finally, LGR1404 causes apoptosis in treated cells, as assessed by activation of caspases, fragmentation of PARP and nuclei condensation. In conclusion, all biochemical and cellular effects of the compound are fully consistent with direct inhibition of CDKs, both cell cycle and transcriptional. The novel prototype inhibitor significantly exceeds activities of roscovitine and, thus, demonstrates the qualities of all other pyrazolo[4,3-d]pyrimidine inhibitors with potential pharmacological applications in oncology.

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Dysregulation of defence systems by 5-fluorouracil in colon cancer HT-29 cells

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A primary cause of cancer treatment failure and patient relapse is an acquired or intrinsic resistance to anticancer therapies. Acquisition of drug resistance can be attributed to various factors including inhibition of apoptosis, altered expression of multidrug resistance-associated proteins, altered drug metabolism or uptake, and/or overexpression of defence systems. Since various anticancer drugs are potential inducers of defence pathways, this could have a marked incidence on cancer cell resistance. Using colon HT29 cells, we found that 5-fluorouracil (5-FU), widely used in the treatment of colorectal cancer, induced the expression of mRNAs encoding glutathione transferases M3 and S1 and antioxidants enzymes such as NAD(P)H:quinone oxidoreductase 1, heme oxygenase-1 and γ-glutamylcysteine synthetase. To further determine the mechanisms involved in 5-FU effects, we investigated whether it activates the Nrf2/antioxidant response element (ARE) pathway which is implicated in the regulation of several genes involved in cell defense systems. Translocation of Nrf2 into the nucleus after 5-FU exposure was demonstrated by immunolocalization and western blot assays. By using an ARE driven-reporter gene (luciferase) assay, activation of the luciferase activity by 5-FU was evidenced and this effect was inhibited by co-transfecting a vector expressing a dominant negative Nrf2. Moreover, transfection of Nrf2 siRNA into HT-29 cells increased 5-FU cytotoxicity. In conclusion, these results demonstrate that 5-FU activates the Nrf2/ARE pathway which modulates the chemosensitivity of colon cancer HT29 cells and might represent a potential therapeutic target in 5-FU treatment.

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Heat shock protein 90 inhibitors modulate choline phospholipid profiles and metabolizing enzymes in human melanoma cells

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Heat shock protein 90 (Hsp90) inhibition is a novel anticancer strategy permitting simultaneous depletion of many oncogenic proteins (eg CRAF & HIF-1α) and many Hsp90 targeted drugs such as 17-AAG and 17-DMAG are now in clinical trial. Here we use magnetic resonance spectroscopy (MRS), a non-invasive technique for studying cell metabolism, to assess whether Hsp90 inhibition in human melanoma cells is associated with metabolic alterations that may serve as biomarkers of target modulation in the clinic.

SKMEL28 human melanoma cells were treated with equipotent concentrations of 17-AAG (100 nM), 17-DMAG (200 nM) or our novel agent CCT018159 (30 microM) for 48h then extracted in methanol, chloroform and water (1:1:1), and aqueous fractions analysed by 31P MRS. Western blotting for expression of CRAF and Hsp70 (known to be induced upon Hsp90 inhibition) was used to confirm drug action.

^{31}P MRS analysis indicated that exposure of cells to 17-AAG resulted in an increase in the level of metabolites involved in membrane phospholipid turnover. Cellular phosphocholine (PC), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE) content increased by ~3, 4 and 2.6-fold respectively (n=4, $p \leq 0.02$). Furthermore, nucleoside triphosphates (NTP) and PC/NTP were also increased by 2 and 1.7-fold respectively ($p \leq 0.049$), concomitant with CRAF depletion and Hsp70 induction.

Similar changes were seen with 17-DMAG (PC, GPC and PC/NTP up by 2.8, 4.8 and ~2-fold respectively) and CCT018159 (PC, GPC & PC/NTP up by 2.4, 3 and 1.4-fold respectively).

We next assessed the effect of Hsp90 inhibitors on the activity of enzymes involved in the breakdown of the major membrane phospholipid phosphatidylcholine (PtdCho). Amplex Red spectrophotometric assay of PtdCho specific phospholipase C (PtdCho-PLC) showed a decrease in the enzyme's specific activity to 45±19% of controls (n=4, p=0.015) in 17-AAG treated cells. Western blotting showed a marked reduction in phosphorylated (activated) cytoplasmic phospholipase A2 (cPLA2) but not total cPLA2 in cells treated with all three inhibitors.

Our results indicate that inhibition of Hsp90 in human melanoma cells results in altered choline phospholipid metabolism that is associated with