428 POSTER

Induction of survivin expression via activation of insulin-like growth factor-1 receptor/epidermal growth factor receptor heterodimer: a novel resistance mechanism of EGFR tyrosine kinase inhibitors in non-small cell lung cancer

F. Morgillo[1], J.K. Woo[1], W.K. Hong[1], F. Ciardiello[2], H.Y. Lee[1]. [1]*The University of Texas M.D. Anderson Cancer Center, thoracic head and neck medical oncology, Houston, Texas, USA;* [2]*Seconda Universita degli Studi di Napoli, Dipartimento Medico-Chirurgico di Internistica Clinica e Sperimentale F. Magrass, Napoli, Italy*

Background: The role of EGFR signaling pathway in tumor progression has long been appreciated. However, the overall response rate to EGFR tyrosine kinases (TKIs) is low and the causes of resistance to these drugs are poorly defined. This study was designed to investigate the mechanisms mediating resistance to the drugs.
Methods: The antitumor activities and action mechanisms of EGFR inhibitors (erlotinib, gefinitib, cetuximab), single or in combination with Insulin-like growth factor-1 receptor (IGF-IR) inhibitors, were assessed *in vitro* in a subset of non-small-cell lung cancer (NSCLC) cell lines by the MTT assay, flow cytometry-based TUNEL assay, anchorage-dependent and -independent colony formation, metabolic labeling, coimmunoprecipitation, and northern and western blot analyses and *in vivo* in animal models. EGFR and IGF-1R expression was assessed in lung tissue samples from patients with NSCLC.
Results: EGFR TKIs inhibited the proliferation and anchorage-dependent and -independent colony-forming abilities of NSCLC cells by inducing apoptosis only when IGF-1R signaling was blocked. Treatment with EGFR TKIs, but not with the EGFR antibody, induced EGFR:IGF-1R heterodimerization on cell membrane and activation of the IGF-1R, resulting in the stimulation of PI3K/Akt/mammalian target of rapamycin (mTOR) pathway, promoting the *de novo* protein synthesis of survivin and EGFR, resulting in the survival of NSCLC cells. Inhibition of IGF-1R activation, suppression of mTOR-mediated protein synthesis, or knockdown of survivin expression abolished resistance to the EGFR TKIs and induced apoptosis in NSCLC cells *in vitro* and *in vivo*. The majority of patients with EGFR-overexpressing NSCLC had correlatively high levels of IGF-1R in tumors compared with those in normal counterparts.
Conclusions: IGF-1R activation interferes with the antitumor activity of EGFR TKIs and IGF-1R expression may serve as a predictor for EGFR TKI resistance in NSCLC. IGF-1R-targeting combination treatment is required when EGFR TKIs are considered as therapeutic strategies for NSCLC patients.

429 POSTER

JG3, a novel heparanase inhibitor simultaneously targeting bFGF, combats tumor angiogenesis and metastasis

H.J. Zhao[1], H.Y. Liu[1,2], Y. Chen[1], X.L. Xin[2], J. Li[2], Y.T. Hou[1], Z.H. Zhang[1], C.Y. Xie[1], M.Y. Geng[1,2], J. Ding[1]. [1]*Shanghai Institute of Materia Medica, Shanghai, China;* [2]*Marine Drug and Food Institute, Ocean University of China, Qingdao, China*

Background: Heparanase has become a tractable and highly attractive target in cancer therapy. A challenge is thus encouraged to develop the appreciable potent heparanase inhibitors with better pharmacological profiles. Here, we report that JG3, a novel marine-derived oligosaccharide, stood out as a potential substrate-based heparanase inhibitor.
Material and Methods: The heparanase activity was determined by FITC-HS-based HPLC chromatography. The binding kinetics profiles and the binding structural motifs were characterized by surface plasmon resonance. The release of bFGF from ECM was determined using ELISA assay. The *in vitro* and *in vivo* angiogenesis was assessed via endothelial cell proliferation and migration, rat aortic ring and chicken chorioallantoic membrane methods. The *in vivo* angiogenesis and metastasis were evaluated in both murine B16F10 experimental lung metastasis model and human breast cancer MDA-MB-435 cells orthotopically xenografted athymic mouse model.
Results: JG3 significantly and concentration-dependently inhibited heparanase enzymatic activity in cell-free system by specifically binding to the KKDC and QPLK epitopes on heparanase, yielding an IC_{50} value of 6.55 ng/ml. In particular, LMW heparin, but not other glycosaminoglycans (GAGs), competitively inhibited the interaction of JG3 with heparanase. Further *in vitro* studies demonstrated that JG3 suppressed heparanse-driven invasion of both NIH-3T3 cells stably expressing heparanase and MDA-MB-435 human breast cancer cells. In addition, JG3 abolished the release of HS-sequestered bFGF from the subendothelial ECM, and repressed its subsequent angiogenesis. Moreover, JG3 was capable of inactivating bFGF-induced FGFR and ERK1/2 phosphorylation, and blocking bFGF-triggered angiogenic events by directly binding to bFGF via heparin-binding domain. Collectively, JG3 combated lung metastasis in a murine B16F10 experimental metastasis model as well as lung metastasis and angiogenesis of MDA-MB-435 orthotopic xenografts in athymic mice, accompanied by a potent suppression of primary tumor growth.
Conclusions: Together, the *in vivo* angiogenesis and metestasis inhibition of JG3 may be the comprehensive reflection of two defined mechanisms but serving the same outcome: namely, JG3 simultaneously blocked heparanase activity as a non-cleavable substrate mimetic of heparan sulfate and limited the availability of HS-binding growth factor bFGF as a competitive inhibitor. These findings favorably suggest that JG3 should be considered as a promising candidate agent for cancer therapy.

430 POSTER

Telomere Damage promotes antitumoral activity of the G-quadruplex ligand RHPS4

B. Benassi[1], E. Salvati[1], C. Leonetti[1], A. Rizzo[1], M. Mottolese[2], M. Stevens[3], M. D'Incalci[4], E. Gilson[5], G. Zupi[1], A. Biroccio[1]. [1]*Regina Elena Cancer Institute, Experimental Chemotherapy Laboratory, Rome, Italy;* [2]*Regina Elena Cancer Institute, Pathology Laboratory, Rome, Italy;* [3]*University of Nottingham, School of Pharmacy, Nottingham, UK;* [4]*Pharmacological Research Institute "Mario Negri", Department of Oncology, Milan, Italy;* [5]*Ecole Normale Supérieure de Lyon, Laboratory of Molecular Biology of the Cell, Lyon, France*

Background: G-quadruplex (G4) ligands were initially designed to counteract telomerase action at telomeres. Surprisingly, their antiproliferative effects can occur in telomerase-negative cells and follow kinetics that cannot be merely explained by telomere shortening, suggesting that these compounds affect other pathways, not necessarily related to telomere biology.
Materials and Methods: TIF (Telomere dysfunction-Induced Foci) index, defined as foci of DNA damage response factors that coincide with TRF1 has been calculated by confocal microscopy using antibodies against endogenous proteins. Antitumoral activity of RHPS4 has been evaluated by i.v. treating xenografted mice with RHPS4 at 15 mg/kg for fifteen consecutive days. *In vivo* pharmacodynamics monitoring of RHPS4 effects has been performed in tumor sections by analysis of telomere length, apoptosis, proliferation and telomere damage response.
Results: We demonstrate that the G4 ligand RHPS4 triggers a rapid and potent DNA damage response at telomeres with the formation of several telomeric foci containing phosphorylated H2AX, Rad17 and 53BP1. This phenomenon is PI3 kinase-dependent, results from delocalization of POT1 and is antagonized by the overexpression of either POT1 or TRF2. *In vivo*, RHPS4 is highly active as a single agent by inducing telomere injury and apoptosis. Tumor inhibition is accompanied by a strong DNA damage response and tumors overexpressing either POT1 or TRF2 are completely resistant to the treatment.
Conclusions: The data reported in this paper provide evidences that the G4 ligand RHPS4 is a telomere damage inducer and that telomere disruption selectively triggered in malignant cells results in a marked anticancer effect. They further validate telomeres as very promising therapeutic targets and identify RHPS4 as a strong candidate for clinical application. The combined use of G4 ligands and TRF2 or POT1 inhibitory molecules may have synergistic effect in tumor response offering new opportunity to cancer therapy.

431 POSTER

Elucidation of additional targets of the thioredoxin inhibitor PMX 464

T.D. Bradshaw, C.S. Matthews, E.-H. Chew, M.F.G. Stevens. *University of Nottingham, School of Pharmacy, Nottingham, United Kingdom*

4-(Benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (PMX 464), a novel antitumour agent discovered at the University of Nottingham, UK and licensed to Pharminox Ltd exhibits potent and selective growth inhibitory effects against certain colon, breast and renal carcinoma models *in vitro* and in *vivo*. PMX 464 targets thioredoxin (Trx), binding the active site cysteine residues and inhibiting protein disulphide oxidoreductase activity dose dependently (IC_{50} 3 μM). Herein we describe experiments undertaken to challenge the selectivity of PMX 464, explore downstream consequences of Trx inhibition and aid further molecular target elucidation of PMX 464.
PMX 464 is not a promiscuous cysteine sulfhydryl inhibitor: in the presence of PMX 464 (1 μM, 10 μM) the activity of ficin (a cysteine protease of the papain family) is not compromized. Indeed, the S-S interatomic distance (3.9 Å) in the Trx active site affords sulphur atoms disposed to attack the electrophilic β-carbon atoms of the cyclohexadienone pharmacophore. Trx is a negative regulator of apoptosis signal-regulating kinase (ASK-1); its overexpression in tumors correlates with tumor aggression and resistance to therapy. Active site cysteines (C32 and C35) of reduced Trx bind to