American Association for Cancer Research

92nd Annual Meeting

March 24-28, 2001 New Orleans, LA

Volume 42 March 2001

Proceedings

IGF-1 stimulated phosphorylation of Erk-1 and Erk-2. The cells were exposed for 2 hours to graded concentrations of PD 98059 (0.1 to 15.0 microM) and stimulated for 5 minutes with IGF-1 (10 ng / mL) and the phosphorylation levels of Erk-1 and Erk-2 were detected by Western blot using phospho-Erk 1 / 2 (p42/44) antibody. At 1microM PD 98059 completely suppressed the activation of Erk-1 and Erk-2. Further, IGF-I-stimulated ERK-1/2 phosphorylation was inhibited for up to 6 days by PD98059 (1 to 15 microM). Similar results were obtained with UO126, another MEK1 inhibitor. Of interest, IGF-I protected against rapamycin in the presence of UO126 or PD98059. These results indicate that IGF-I-mediated rescue from rapamycin-induced apoptosis, and growth inhibition is independent of both mTOR and ERK-1/2. To elucidate the pathway(s) involved we have started to analyze changes in transcription induced by IGF-I in the presence of rapamycin plus PD98059. Initial results using an Affymetrix HG-U95A Gene Chip (12,559 human genes), indicate that expression of relatively few genes change following 1 hr IGF-I stimulation (0.65% > 2-fold: 19 increased, 65 decreased). Supported by CA23099, CA77776 and by ALSAC.

#4303 The PKC Activator Bryostatin 1 Potentiates Ara-C-Induced Apoptosis in Human Leukemia Cells Overexpressing Bcl-Xl by Promoting Cytochrome C Release. Zhiliang Wang, Shujie Jenny Wang, and Steven Grant. *Medical College of Virginia, Richmond, VA.*

We previously reported that in U937 cells, the macrocyclic lactone bryostatin 1 (Bry) partially overcame resistance to ara-C-mediated apoptosis conferred by overexpression of Bcl-2 (Wang et al., Mol Pharm 52:1000, 1997). The purpose of the present studies was to extend these findings to the anti-apoptotic protein Bcl-xL, the cytoprotective effects of which have been shown to differ from those of bcl-2, and to explore mechanisms by which enhanced apoptosis might occur. Enforced expression of bcl-xL markedly reduced ara-C (1 μM; 6 hr)-induced cytochrome c release, loss of $\Delta\Psi$m, procaspase-3 cleavage, and PARP degradation. Subsequent exposure of U937/Bcl-xL cells to bry (10 nM; 18 hr) partially restored ara-C-induced cytochrome c release, loss of $\Delta\Psi$m, caspase-3 and -9 activity and apoptosis. While the caspase inhibitor ZVAD-fmk blocked ara-C/Bry-induced apoptosis and loss of $\Delta\Psi$m in U937/Bcl-xL cells, it failed to oppose cytochrome c release, suggesting the latter represents a primary event in potentiation of apoptosis. Bry did not alter levels of apf-1, nor did it increase the amount of Bcl-xL co-immunoprecipitating with this protein. Exposure of U937/Bcl-xL-overexpressing cells to Bry led to alterations in the mobility of Bcl-xL following PAGE analysis, manifest by a broadening of the ectopically expressed Bcl-xL protein band. Lastly, subsequent exposure of U937/Bcl-xL cells to ara-C → Bry reduced colony formation to levels observed in wild-type cells exposed to ara-C alone. These findings indicate that Bry partially restores ara-C sensitivity in cells ectopically expressing Bcl-xL through a mechanism that involves potentiation of cytochrome c release.

#4304 In-Vitro Effect of the Epidermal Growth Factor Receptor Inhibitor PKI 166 on Human Lung Cancer Cell Lines. Kil Dong Kim, Rachel Zuei T. Tsang, Waun Ki Hong, and Roy S. Herbst. *The University of Texas M. D. Anderson Cancer Center, Houston, TX.*

Epidermal Growth factor receptor (EGFR) is a member of a family of four growth factor receptors [EGFR(HER1 or ErbB1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4)]. These receptors are large proteins (~178KD) which reside in the cell membrane, each with a specific external ligand binding domain. High EGFR expression has been observed in 75% of lung cancer patients and hence represents an excellent target for therapy. We investigated EGFR expression of the Non-small cell lung cancer (NSCLC) cell lines, H226 (squamous), H322 (Bronchoalveolar,BAC), A549 (adenocarcinoma), H1299 (large cell), H358 (BAC.), H460 (large cell.), H596 (adenosquamous), H522 (adenocarcinoma.), PC14PE6 (adenocarcinoma), H1792 (adenocarcinoma H292 (mucoepidermoid), H157 (squamous) and DMS-4C(adenocarcinoma.) using western blot analysis. The H226, H322, H358, A549, H292 and H157 cell lines demonstrated increased EGFR expression. H226 had the strongest expression of EGFR (+++), while the others had less but measurable expression: H157 (++), H292 (++), H322 (++), H358 (+) and A549 (++). We next investigated these highs expressing cell lines for activated EGFR activity using an anti-phosphotyrosine antibody with and without exogenous EGF stimulation and studied the ability of PKI 166, a small molecule, oral EGFR tyrosine kinase inhibitor to block this process. The concentration of PKI 166 used ranged from 0 to 2.5μM. In the cell lines absent EGF stimulation, there was minimal phosphorylated tyrosine kinase activity observed at baseline. In the cell lines with EGF stimulation, the signal intensity of phosphorylated tyrosine kinase was enhanced and decreased in the immunoprecipitation study with the addition of PKI-166. The cell lines were differentially inhibited by PKI-166: H226 (–), H157 (—). H292 (—), H322 (–), H358 (-), A549 (-). In most cases, this inhibition correlated with the baseline level of EGFR. These results demonstrate the potential use of PK1-166 in in-vivo studies and therapeutic animal models are currently underway.

#4305 Deregulated P13k/AKT/TOR Pathway in PTEN-Deficient Tumor Cells Correlates with an Increased Growth Inhibition Sensitivity to a TOR Kinase Inhibitor CCI-779. K. Yu, W. Zhang, J. Lucas, L. Toral-Barza, R. Peterson, J. Skotnicki, P. Frost, and J. Gibbons. *Department of Oncology/Immunoinflammation, Wyeth-Ayerst Research, Pearl River, NY.*

Deregulation of the P13K/AKT/TOR signaling pathway is widely believed to play a major role in human cancer. Genetic deletion or mutation of the PTEN/MMAC1 tumor suppressor, a dual specificity phosphatase and a critical negative regulator of this pathway, is estimated to occur in as many as 50% of all solid human tumors. CCI-779, a macrolide inhibitor of the TOR kinase, is presently undergoing clinical cancer trials. The mode of action by CCI-779 suggests that the drug will be an effective therapy for human tumors, and it may demonstrate an even greater activity against tumor cells with elevated AKT/TOR signaling caused by cellular alterations, including the loss of PTEN tumor suppressor. We have conducted a survey of the levels of the phophorylated (active) AKT in a panel of brain, prostate, breast and colon tumor cell lines. The phospho-AKT status in these cells were further compared with their growth inhibition sensitivity to CCI-779. We found that in serveral tumor types, loss of PTEN tumor suppressor and/or activation of AKT correlated directly with an increased susceptibility to CCI-779. In most cases, tumor cells that are resistant to CCI-779 are found to have low or a moderate level of activated AKT. These in vitro cell culture observatons have been further supported by the activity of CCI-779 against the growth of human tumors implanted in nude mice. Furthermore, several cell lines that contain a moderate level of activated AKT are also sensitive to CCI-779. Our data indicate that an increased phosphorylation of AKT renders cells more sensitive to the inhibition, but it is not a requirement for the inhibition by the drug.

#4306 ZD1839 ('Iressa'), an EGFR Tyrosine Kinase Inhibitor, Potentiates Non-Mhc Restricted Cytotoxicity in Human Cancer Cell Lines. Alfredo Budillon, R. Guarrasi, E. Di Gennaro, F. Bruzzese, S. Errico, G. Pirozzi, M. Caraglia, A. Avallone, P. F. Tassonel, M. L. Lombardi, F. Caponigro, S. Venuta, and P. Tagliaferri. *Magna Graecia University, Catanzaro, Italy, and National Cancer Institute - G. Pascale, Napoli, Italy.*

Disregulated expression or function of the growth factor receptors of the erbB2 family is a common finding in several human tumors. The growth factor mediated-signalling has been considered to promote cell proliferation and also to behave as a survival, proangiogenic and antiapoptotic pathway. Overexpression and functional alterations of erbB family members has also been correlated to resistance of tumor cells to non-MHC restricted cytotoxicity. We have previously shown an increased resistance to Lymphokine activated Killer (LAK)-mediated cytolysis in immortalized MCF-10 breast cells overexpressing erbB2 (P. Tagliaferri et al CCR, 1996). In addition, using a cold target competition assay, we have demonstrated that resistance to LAK is not due to altered effector/target binding, suggesting that the activation of growth factor signalling induces resistance to immune cytolysis acting downstream to the lymphocyte recognition of tumor cells. On these bases, we have investigated whether ZD1839 ('Iressa'), an orally active, selective EGFR tyrosine kinase inhibitor (EGFR-TKI), may increase the sensitivity of tumor cell lines to non-MHC restricted cytotoxicity. We have found that untreated HNSCC and Melanoma cells expressing high levels of EGF-R and of other erbB receptors were almost completely resistant to immuno-cell killing but showed increased sensitivity to LAK-mediated cytotoxicity, as demonstrated by LDH-specific release assay, after ZD1839 treatment. In particular, we have demonstrated a dose dependency (treatment range 0.1-5 μM ZD1839) which was already evident after 6 h treatment and decreased after 48 h. Induction of sensitivity to activated lymphocytes occurred in parallel to downregulation of ERK-1/ERK-2 activity and could be detected when cells had not yet lost full viability. These results suggest that EGFR mediated signalling could represent a useful cellular target for overcoming tumor cell resistance to activated lymphocytes and provide a potential rationale for the clinical use of the EGFR tyrosine kinase inhibitor ZD1839 ('Iressa') in combination with immunostimulating cytokines. 'Iressa' is a trade mark of the AstraZeneca group of companies.

#4307 Epidermal Growth Factor Receptor Tyrosine Kinase Inhibition by ZD1839 ('Iressa') Blocks Oestrogen-Stimulated Proliferation and Progesterone Receptor Expression in Human Breast Epithelium. Kai C. Chan, W. Fiona Knox, R. Dobson, C. S. Potten, and N. J. Bundred. *University Hospital of South Manchester, Dept. of Surgery, Manchester, UK, and University Hospital of South Manchester, Dept. of Surgery, Manchester, UK.*

Normal breast epithelium commonly expresses epidermal growth factor receptor (EGFR) and oestrogen receptor. Several in vitro studies suggest that oestrogen stimulates proliferation by utilising the EGFR pathway. We sought to determine if ZD1839 ('Iressa'), an EGFR tyrosine kinase inhibitor, decreases proliferation and progesterone receptor (PR) expression in normal breast epithelium. Premenopausal normal breast tissue from 8 women was implanted subcutaneously into athymic nude mice in separate experiments (8 xenografts per mouse; median number of mice used per experiment = 14). Following 14 days, each mouse had two xenografts removed and a 2 mg oestradiol (E2) pellet inserted subcutaneously, following which the mice were treated daily with ZD1839 (10-100 mg/kg) or vehicle for 14 days. The remaining xenografts were removed after 7 and 14 days of treatment. Proliferation and progesterone receptor (PR) expression were assessed by determining the percentage of positive cells in 1000 epithelial cells counted following immunostaining for Ki67 (proliferative marker) and PR, respectively. After 14 days' treatment, there was a rise in median proliferation and PR expression in the vehicle + E2 group compared with the pre-treatment level (5.4 % vs 2.6 %, and 26.2 % vs 14.7 %, respectively; both $p<0.001$). In the ZD1839 + E2 group there was a decrease in epithelial proliferation and a decrease in PR expression compared with the control vehicle + E2 group (1.1 % vs 5.4%, and 20.1 % vs 26.2%, respectively, both $p<0.001$). These results suggest that oestrogen-stimulated proliferation and PR expression do