

two models of highly resistant lung cancer cells: 1) Adherent cells (anchorage-dependent) growing under prolonged periods of serum starvation (PPSS) and 2) cells growing as floating (anchorage-independent) tumorspheres (FTs) to evaluate the effect of REV 5901. Cell viability was determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots. Compared to cells growing under routine culture conditions (RCCs), cells growing under PPSS or as FTs were highly sensitive to REV. REV was able to selectively and irreversibly decrease the viability of cells growing under PPSS or as FTs within 24 h. Recovery experiments exposing cells to REV for 24 h followed by incubation in drug-free media for 48 h demonstrated that while PPSS as well as FTs cells were unable to recover, the noncancerous cell line Beas-2B growing under RCCs was not only less sensitive to REV but was also able to recover significantly. At the molecular level, REV induced significant changes in the expression of key proteins of the Wnt signaling pathway. Our data demonstrate that short treatment with REV can eliminate highly resistant cancer cells and that the Wnt signaling pathway may play a central role.

B34

Combination Therapy with Wnt Pathway Modulators to Override Chemoresistance in Human Lung Cancer Cells



J.S. Yakisich, V. Kaushik, A.R. Guishard, D. Afolabi, N. Azad, A.K.V. Iyer Hampton University, Hampton, VA/US

Background: The serum levels of DKK1, a negative regulator of the Wnt signaling pathway, have been reported to be elevated in cancer patients. DKK1 expression and association to chemoresistance has not been extensively investigated in cancer stem-like cells. In this study, by using cancer cell lines growing under anchorage-dependent conditions (Adherent cells; chemosensitive phenotype) as well as cells growing under anchorage-independent conditions (Floating Spheroids (FSs); chemoresistant phenotype), we evaluated a) the expression of DKK1 and the downstream effector of the Wnt signaling pathway β -catenin and b) the effect of iCRT-14 (a β -catenin inhibitor) and WAY-262611 (a DKK1 inhibitor) on the viability of cancer cells. **Methods:** FSs were grown in ultra-low attachment plates for 7 days. Cell viability were determined by the MTT or the CCK assay for adherent cells and FTs, respectively. Protein levels were determined by Western blots. **Results:** A549 and H460 adherent cells were sensitive to both iCRT-14 and WAY-262611. FSs generated from these cell lines were resistant to WAY-262611 but still sensitive to iCRT-14. FSs prepared from H460 cells were more sensitive to iCRT compared to FSs prepared from A549 cells. Western blot analysis from protein lysates prepared from H460 cells showed that iCRT-14 decreased the expression of β -catenin. **Conclusions and Future Directions:** Our data demonstrate that a DKK-1 inhibitor in combination with a β -catenin inhibitor has the potential to eliminate lung cancer cells displaying varying degrees of chemoresistance. We are currently characterizing the mechanism by which this combination modulates the Wnt signaling pathway.

B35

Circulating Tumor-Associated Cells in Lung Cancers Are Resistance-Educated per Previous Chemotherapy Treatments



D.B. Akolkar,¹ S. Limaye,² D. Patil,¹ S. Patil,¹ V. Mhase,¹ S. Apurwa,¹ S. Pawar,¹ V. Todarwal,¹ V. Datta,¹ C. Sims,¹ A. Srinivasan,¹ R. Datar¹ ¹Datar Cancer Genetics Limited, Nasik, Maharashtra/IN, ²Kokilaben Dhirubhai Ambani Hospital, Mumbai/IN

Resistance to chemotherapy agents is frequently encountered in non-small cell lung cancers (NSCLC) and is largely undetected

until symptomatic or radiologic detection of disease progression. Real-time monitoring of chemoresistance in NSCLC is an unmet clinical need. We describe a novel approach for real-time chemoresistance profiling (CRP) in NSCLC using peripheral blood circulating tumor-associated cells (CTACs), which are apoptosis-resistant cells of tumorigenic origin (EpCAM+, pan-CK+, CD45±). Peripheral blood was collected from 145 patients with confirmed NSCLC including 102 therapy-naïve cases and 43 pretreated cases. Peripheral blood mononuclear cells (PBMCs) were harvested by centrifugation. C-TACs were enriched using an epigenetically activated medium that eliminates normal (nontumorigenic) cells and confers survival privilege on apoptosis-resistant tumorigenic cells (C-TACs). Surviving C-TACs were confirmed by immunostaining (EpCAM, pan-CK, CD45, TTF-1, Napsin-A). Harvested C-TACs were treated in vitro with a panel of conventional cytotoxic agents and the fraction of surviving cells estimated to determine resistance profiles. Among the therapy-naïve NSCLC, innate chemoresistance towards any agent was observed in 51.7% of cases, which included resistance towards platinum agents in 37.8% of cases, microtubule targeting agents in 54.5% of cases, antimetabolites in 57.1% of cases, and topoisomerase inhibitor in 57.3% of cases. Among the pretreated NSCLC cases, resistance towards any agent was observed in 88.1% of cases, which included resistance towards platinum agents in 84.9% of cases, microtubule targeting agents in 85.1% of cases, antimetabolites in 96.7% of cases and topoisomerase inhibitor in 100% of cases, respectively. In vitro chemoresistance profiling of C-TACs is a viable approach for real-time monitoring of innate and acquired chemoresistance. Higher chemoresistance in the pretreated population, as compared to the therapy-naïve population, indicates that C-TACs are resistance-educated by prior treatments.

B36

Effects of Trifluoperazine and Its Analog on A549 Human Lung Cancer Cells



J. Jeong, J. Park, N. Park, G. Kang, S.S. Kang Gyeongsang National University, Jinju/KR

Although there have been great advances in technology, molecular diagnosis, and therapeutics, lung cancer is still the leading cause of cancer-related mortality all over the world. Recently, some antipsychotic drugs have been shown to possess anticancer activity. Thus, the present study was designed to evaluate the anticancer effects of trifluoperazine (TFP), a commonly used antipsychotic drug, and its synthetic analogs on human lung cancer cell lines. To this end, effects of TFP and its selected analog on A549 cells were investigated in vitro as well as in vivo experiments. Synthetic TFP analogs were evaluated by the proliferation of A549 cells following drug treatment and compared to TFP. 3dc, a selected TFP analog, significantly inhibited the proliferation of A549 cells. Further experiment showed that TFP and 3dc had activities to inhibit the anchorage dependent/independent colony formation, and migration of A549 cells. Western blot analysis revealed that 3dc affected the gene expression levels related to apoptosis and cell cycle. Flow cytometric analysis showed that 3dc induced sub-G1 and G1 population and reduced cell population in S and G2/M phase. Additionally, Annexin V/PI staining showed that 3dc increased apoptotic cell population. Moreover, 3dc increased DNA fragmentation. 3dc showed stronger anticancer effects in all the experiments than TFP. In addition, in in vivo experimental models, 3dc also showed powerful anticancer effect in orthotopic lung cancer development than TFP. Thus, the present study demonstrates that a synthetic TFP analog has anti-lung cancer activity and provides a potential therapeutic candidate for lung cancer.