to cytotoxic and immunotherapies. The precise molecular details for how interactions between individual components of the tumor microenvironment impact cancer progression and metastasis are not well understood. Elucidating complex interactions within the tumor microenvironment is essential for identifying novel therapeutic targets but has proved challenging because isolating and studying cancer-associated stromal cells from primary lung tumors has remained difficult. To begin to fill this knowledge gap, we developed a rapid, reliable, and reproducible culture system that allows for the isolation and expansion of large quantities of primary cancer-associated mesenchymal (CaM) cells. Briefly, by combining fibroblast-derived extracellular matrices with hypoxic culture conditions, we developed a microenvironmental mimetic cell culture system. Primary lung cancer biopsies are dispersed and put into this system, and the resulting cells that expand are CaM cells with stem-like properties. We show that CaM cells and their deposited extracellular matrices alter signaling pathways and migration of lung cancer cells in vitro, and when co-injected with lung cancer cells, CaM cells induce metastasis in vivo. Thus, the overall hypothesis of this work is that CaM cells drive lung cancer metastasis by reprogramming the extracellular milieu and inducing metastatic signaling within the cancer cells. We are now working to determine the specific mechanisms by which primary CaM cells influence metastatic phenotypes of cancer cells. Further, these experiments will lead to novel therapeutic targets by identifying interactions and signaling events that are only initiated by CaM cells and their deposited ECM within the tumor microenvironment.

A16 Autoantibody-Antigen Complexes Can Detect Limited-Stage Small-Cell Lung Cancer

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Small-cell lung cancer (SCLC) claims 30,000 American lives each year with five-year survival rates of just ~7%. Somewhat lost in these dismal statistics is the fact that patients diagnosed early (limited stage) display vastly superior survival metrics when compared to those diagnosed late (extensive stage). Since nearly 20% of limited-stage SCLC can be cured with conventional cytotoxic chemotherapy and/or surgery, earlier diagnosis could have a clinical impact. Unfortunately, the computed tomography screening approaches capable of early detection for non-small cell lung cancer (NSCLC) have not proven effective for SCLC. We have found that overall levels of plasma autoantibody-antigen complexes are >2x higher in SCLC compared to other cancer types including NSCLC, colon, breast, and pancreas cancer. Thus, we hypothesized that a blood-based autoantibody test could reliably detect SCLC while still at limited stage. Using high-density antibody arrays, we discovered and twice validated 8 IgG and 11 IgM highly specific autoantibody-antigen complexes for SCLC in 3 independent cohorts (1 pre-diagnostic and 2 diagnostic, total N=240). Using optimized logistic regression, we identified 4 autoantibody-antigen complexes that performed well in each study with an AUC of 0.915 (53% sensitivity at 90% specificity) in the pre-diagnostic set, 1.0 (100% sensitivity at 90% specificity) in the first diagnostic cohort and 0.866 (64% sensitivity at 90% specificity) in the second diagnostic cohort. Panel autoantibodies were similarly effective when the plasma was drawn up to 1 year prior to diagnosis, at limited-stage diagnosis, or at extensive-stage diagnosis. We have evidence that each panel autoantibody is specific for SCLC as none are upregulated in NSCLC (N=45) samples or in other comorbidities examined, including COPD (N=31) and autoimmunity (N=15). Our findings suggest these autoantibodies have the potential to be used at the time of lung cancer screening to identify limited-stage SCLC to increase the survival of this recalcitrant cancer.

A17 Inhibition of RUVBL1/2 ATPase Activity Drives Immune Infiltration and Radiosensitizes Non-Small Cell Lung Cancer

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Purpose of Study: Prior work in non-small cell lung cancer has shown that RUVBL1 and RUVBL2 (herein RUVBL1/2) are overexpressed in patient tumors and high expression predicts poor patient prognosis. Additionally, the inhibition of RUVBL1/2 ATPase activity using small molecules has shown modest activity as a monotherapy in some preclinical models. In this study we evaluated what clinically relevant agents could be combined with RUVBL1/2 inhibitors to improve therapeutic efficacy in NSCLC. Experimental Procedures: Patient-derived NSCLC lines were treated with radiation, chemotherapy, and targeted inhibitors in combination with a RUVBL1/2 inhibitor, and viability was measured to determine synergy/potentiation using Combenefit (Bliss and Loewe synergy). Both the kinetics and magnitude of DNA damage after RUVBL1/2 inhibition and radiation was measured using immunofluorescence and Western blot in NSCLC and normal human bronchial epithelial cells. Humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor, and intratumoral immune infiltrate was measured using flow cytometry. Activation of the cGAS/STING pathway was monitored after RUVBL1/2 inhibitor treatment in NSCLC lines using Western blot. Summary: Patient-derived NSCLC lines were treated with clinically relevant chemotherapies, targeted inhibitors, or radiation in combination with a highly specific RUVBL1/2 inhibitor or its enantiomer control and cell viability was measured. RUVBL1/2 inhibition significantly enhanced the killing of NSCLC following radiation, but not chemotherapy or other targeted agents. This enhancement was specific to NSCLC, not normal bronchial epithelial cells, and occurred by inhibiting the ability of NSCLC to efficiently repair their DNA. To gauge the effect of RUVBL1/2 inhibitors on the immune system, and thus potentially immunotherapy, humanized mice bearing NSCLC xenografts were treated with a RUVBL1/2 inhibitor. Treatment with a RUVBL1/2 inhibitor caused infiltration of T cells, B cells, and dendritic cells, suggesting that RUVBL1/2 inhibition stimulated the immune system. Additionally, treatment of NSCLC lines in vitro with a RUVBL1/2 inhibitor and radiation activates the cGAS/STING pathway, suggesting that RUVBL1/2 inhibitors could be combined with radiation and immunotherapy.