level, with copy number gain and/or promoter hypomethylation occurring in 50-60% of tumors. Mutation was not a frequent event. Importantly, the ELF3 locus at 1q32.1 was focally amplified in 14.0% of tumors (q value=4.44E-05), compared to recently described driver amplifications at MET (7.1%) and ERBB2 (8.2%). DNA-level selection of ELF3 was observed in 56% of tumors with no known driver, and was statistically enriched in KRAS mutant tumors and patients who smoked. In vitro knock-down of ELF3 in AC cell lines lead to significantly reduced proliferation and fewer colonies formed, while overexpression of ELF3 in HBECs lead to significantly increased proliferation. ELF3 overexpression alone was not sufficient to transform HBECs. In vivo, tumors with knock-down of ELF3 displayed a trend towards decreased growth over time (p=0.08). ELF3 localization was found to be nuclear in both cell lines and tumor specimens, consistent with its function as a transcription factor.

Conclusions: Amplification and hypomethylation of the ELF3 locus is sufficient to de-couple the dependence of ELF3 overexpression on SMAD4 loss. Furthermore, ELF3 is a novel candidate oncogene on chromosome 1q, a region that has remained "driverless" for some time. Focal amplification of ELF3 occurred at a higher frequency than MET and ERBB2, recently described drivers of lung AC, while additional DNA-level mechanisms of selection occurred in over half of cases with no known driver. Manipulation of ELF3 levels in cell models demonstrated a clear role in the regulation of proliferation; further studies are warranted to determine the potential of ELF3 as a pharmaceutical target.

Detection of a novel RET gene fusion in a non-small cell lung cancer patient using AMP chemistry

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Introduction: In lung cancer, several driver Receptor Tyrosine Kinases (RTKs) have been identified in gene fusions, including ALK, RET, ROS1, NTRK1, NRG1 and FGFR1/2/3. These oncogenic gene fusions have become either direct targets or biomarkers for molecular therapies for patients with lung cancer. Screening patients for the presence of these gene fusions would be critical for oncologists to select appropriate therapeutic intervention or for drug developers to design novel therapeutics and conduct clinical trials. However, detection of fusions, especially unknown fusions, from FFPE samples is challenging for many diagnostic techniques. Archer FusionPlex Lung Thyroid Panel is built on Anchored Multiplex PCR (AMP) Chemistry, allowing detection of both novel and known fusions. Here we report the results from our study using the Archer Lung-Thyroid Panel. We identified and verified the presence of a novel fusion between Myosin Phosphatase Rho Interacting Protein (MPRIP) and RET oncogene, in an FFPE sample from a non-small cell lung cancer (NSCLC) patient.

Methods: FFPE samples from 20 NSCLC patients were analyzed in the study. An RNA sample containing an ALK and ROS1 fusion was used as the positive control, and FFPE processed GM12878 was used as the negative control. In addition, FFPE controls containing TMP3-NTRK1 and CCDC6-RET were also used to evaluate the performance of the panel to detect fusions. Total nucleic acid (TNA) was extracted from the FFPE sections using the AgencourtTM FORMAPURE™ kit. For each sample, 200 ng of TNA was used for library preparation. The barcoded libraries from 14 samples were pooled and sequenced on an Illumina MiSeq using a v2 flow cell. The FASTA files from the MiSeq were then analyzed by the ArcherDX data analysis pipeline to determine the presence of gene fusions with driver genes including ALK, FGFR3, MET, NTRK1, NTRK3, PPARG, RET, and ROS1. The detected fusions in clinical samples were further confirmed by Sanger sequencing and FISH.

Results: The known fusions, including EM4-ALK, SLC34A2-ROS1, TMP3-NTRK1, and CCDC6-RET were successfully detected in the positive RNA and FFPE control samples. For all of the clinical FFPE samples, one 10 micron section was sufficient to yield more than the required 200 ng TNA for library preparation. Although three of the twenty (15%) FFPE samples failed the initial sample quality metrics, libraries were prepared for all 20 NSCLC FFPE samples and sequenced on MiSeq. Among the three samples that did not pass the initial sample QC, two of them also failed the QC metrics for fusion detection. From the seventeen samples that did pass the initial QC, one sample failed the fusion QC. Overall, the success rate for the clinical samples tested with this panel was 85%. Among the 20 NSCLC samples, one sample was found to harbor a novel, in-frame fusion between exon 19 of MPRIP and exon 12 of RET. This type of fusion would retain an intact kinase domain (exons 12-18) of RET, and would presumably be oncogenic. Using the RNA portion of the TNA extracted, cDNA was
The transcription factor Slug induces diverse malignant phenotypes in models of established lung cancer and pulmonary premalignancy

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Inflammation often characterizes the pulmonary tumor microenvironment, as does overexpression of the transcriptional repressors of E-cadherin (e.g., Snail and Slug). While chronic inflammation is now associated with increased lung cancer incidence, Snail and Slug are still best known for their induction of epithelial-mesenchymal transition (EMT) and their contribution to the progression of established lung cancer. Our bioinformatics analysis of lung TCGA data suggests that Slug is among the most impactful of all the transcriptional repressors on patient survival. Therefore, we are now exploring the scope of malignant phenotypes and mechanisms induced by Slug expression in a non-small cell lung cancer (NSCLC) model. We discovered that NSCLC cells exposed to the prototypical inflammatory mediator IL-1B respond with downregulation of epithelial markers (E-cadherin and cytokeratin 18) and upregulation of mesenchymal markers (N-cadherin and vimentin), with the repression of E-cadherin by IL-1B being Slug-dependent. NSCLC cells exposed to IL-1B also demonstrate altered cellular morphology, diminished capacity to form clusters in a 3-dimensional (3D) spheroid model, and increased motility. Using chemical inhibitors of the JNK, MEK/ERK, p38 MAPK, and NF-κB pathways, we determined that JNK and MEK/ERK mediate IL-1B induction of EMT in lung cancer cells. Furthermore, siRNA-mediated knockdown of the Fra-1 component of the AP-1 transcription factor abolished the impact of IL-1B on Slug and E-cadherin in this model, demonstrating a mechanistic link between inflammation and Slug-dependent progression of established lung cancer. Because we have identified a critical role for Snail in lung cancer initiation, we next investigated the contribution of Slug to early lung cancer development. Using human bronchial epithelial cells (HBECs) engineered to express Slug to model pulmonary premalignancy, we observed a diverse array of potentially malignant phenotypes, including EMT, increased production of the pro-angiogenic chemokine CXCL8, enhanced invasion in a 3D air-liquid interface model, and anchorage-independent growth in vitro. Furthermore, we determined that the Slug-driven transformation observed in vitro was not contingent upon an altered proliferation rate, but was more likely related to Slug-driven disruption of stem cell signaling programs. Taken together, our data suggest that Slug may be important in the setting of lung carcinogenesis and that its impact on carcinogenesis extends beyond its repression of E-cadherin. Our data also suggest that Slug may play an important role in the initiation and progression of early stage NSCLC.

Structural analysis identifies an orally active PCNA inhibitor that inhibits the growth of small cell lung cancer cells without causing significant toxicity to nonmalignant cells

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Playing a central role in regulating DNA synthesis and repair, proliferating cell nuclear antigen (PCNA) is indispensable to cancer cell growth and survival. It, therefore, represents a potential molecular target to develop broad-spectrum anti-cancer agents. We discovered a cancer-associated isoform of PCNA (caPCNA), which is ubiquitously and highly expressed in a broad range of cancer cells and tumor tissues. In contrast, this PCNA isoform is not significantly expressed in nonmalignant cells. The secondarily modified region distinguishing caPCNA from normal PCNA expressed in non-malignant cells lies between L126 and Y133 within the interconnector domain of PCNA known to be a major binding site for many of PCNA’s interacting proteins. A cell permeable peptide containing the L126-Y133 sequence blocks PCNA interaction, interferes with DNA replication and homologous combination mediated DNA repair, and induces apoptosis in cancer cells. In contrast, this peptide causes no significant toxicity to normal cells.