Functional lung adenocarcinoma risk SNPs identified through positional integration with human alveolar epithelial cell epigenomes

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Motivation: Lung cancer is the leading cause of cancer death, and lung adenocarcinoma (LUAD) is its predominant histological subtype. Fifteen single nucleotide polymorphisms (SNPs) have been significantly associated with LUAD risk in genome-wide association studies (GWAS). However, because most GWAS SNPs reside in non-coding regions and are co-inherited with hundreds of SNPs in linkage disequilibrium (LD), which SNPs play a causal role in disease development remains largely unknown. We hypothesized that some of these SNPs affect oncogenic transcriptional programs by modulating the activity of gene enhancers in alveolar epithelial cells (AECs), the purported cells of origin for LUAD.

Methods: To test this hypothesis, we overlaid epigenomic features of primary human AECs over the locations of index LUAD risk SNPs and associated high LD SNPs. Luciferase assays for enhancer activity were performed for candidate SNPs that were predicted to disrupt transcription factor (TF) binding sites in loci marked by features of active enhancers. Expression quantitative trait loci (eQTL) analysis was also performed using The Cancer Genome Atlas (TCGA) dataset and the online Genotype-Tissue Expression (GTEx) Portal to identify potential target genes of each SNP-enhancer pair.

Results: Thirty-three LUAD risk-associated SNPs mapped to putative AEC enhancer regions. TF binding site prediction suggests that numerous SNPs might alter the binding affinity of TFs implicated in lung cancer, including RXRA and NKX2-1. Luciferase assays indicate that two of the SNPs significantly affect enhancer activity in lung cancer cell lines. eQTL analyses link each of the putative enhancers to candidate target genes, including both known oncogenes and genes not previously associated with lung cancer.

Conclusions: Taken together, our analyses provide new mechanistic insight into long-known associations between non-coding SNPs and LUAD outcomes, and may ultimately yield more effective and personalized strategies for lung cancer risk assessment, prevention, and treatment.

Magnetic nanocubes for selective capture of circulating tumor cells in NSCLC

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Non-small cell lung cancer (NSCLC) is the leading cause for cancer related mortality rates in the US, often associated with 20-35% response rate and a ~10 month median survival time. Currently, tissue diagnostics is performed using immunohistochemistry, FISH, and PCR for staging and treatment planning. In vivo imaging such as PET or CT is also used to detect the severity of NSCLC. NSCLC metastasize by spreading primary tumor cells to distant organs. Therefore, it is possible to isolate circulating tumor cells (CTC) in patients’ blood, and as the cells originate from tumor, detailed genetic evaluation about the tumor could be performed. Isolation and study on circulating tumor cells is slowly evolving as liquid biopsy of cancer. In fact, CTC detection technique is emerging as prognostic markers to identify treatment response in NSCLC patients. The current CTC capture methodology involves cell search technologies, predominantly relying on EpCAM expression based detection. However with the discovery of tumor heterogeneity and consequent impact on clinical treatment, it is important to detect patients with CTCs early on, based on their genetic alterations. Both HER2 (2-5% mutation incidence) and EGFR (10-35% mutation incidence) overexpression have been pronounced in patient biopsies and their exclusivity in individuals has been seen as a prerequisite in chemotherapeutic selection and dose. Moreover there is no standardized process to selectively identify CTCs based on HER2 and EGFR surface expressions. While EGFR and resistant mutations has been prominent, in the understanding of NSCLC characterization, HER2 is relatively less explored and frequently associated with breast cancer detection. Recent studies show that HER2 expressions correlate with metastases and disease free survival. Overall this fact leads us to believe HER2 markers could be present in CTCs. Therefore, we are developing magnetic iron nanocubes (FeNC) functionalized with either Herceptin or Cetuximab as markers. These CTC markers can be correlated with tumor heterogeneity and decide therapeutic targets for first line and second line treatment. Our approach involves cell sensing using magnetic nanoparticles (MNP), counting and subsequent separation of live A549 (HER2 +ve; EGFR +ve) and HCC827 (HER2 -ve; EGFR +ve) cells from a mixture for further processing. In our current work we have synthesized HER2 and EGFR receptor targeting iron oxide nanoparticles (50nm) that
are highly magnetic, and stable in serum solutions for extended periods of time. The MNPs are incubated with cells in 1X PBS for 3h for receptor binding at 37°C after which particles bound to cells are magnetically separated with a pull force of 57 lbs. Cells are then washed and counted using an automated algorithm. The whole process has been optimized to a minimum cell population of 100 and can be theoretically reduced to a 2h process which makes this extremely effective for clinical evaluations and straightforward. Similar protocol was used when the cells were spiked in blood plasma and captured. Our data suggests strong correlation between number of A549 cell captured when Herceptin conjugated MNPs are used (96% difference vs HCC827), while Cetuximab conjugated MNPs pull both HCC827 as well as A549 (31% difference). We expect to reduce the cell capture limit to less than 10 cells in further experiments as required for patient testing. In conclusion, our results show that MNP based sensing allows both cell marker characterization as well as capture simultaneously. The nanocubes allow better characterization of HER2 and EGFR positive metastatic cell subpopulations and provide easier prediction of tumor heterogeneity without resorting to invasive procedures.

**Targeted nanoconjugate co-delivering siRNA and tyrosine kinase inhibitor to Kras mutant NSCLC reveals Gab1 assisted survival pathway post oncogene knockdown**

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NSCLC is diagnosed in an estimated 220,000 patients each year with five-year overall survival rates of 16 percent. A recent report confirmed that 16 percent of NSCLC patients carry oncogenic KRAS mutation. Patients with KRAS mutation often harbored in wild-type EGFR tumors, are resistant to Tyrosine Kinase Inhibitors (TKI). A potent drug targeted against KRAS mutation has not yet been developed and the objective response rate with the current standard of care is a meager three percent. Interestingly, KRAS knockdown using siRNA sensitizes tumor to TKI with a good response rate. Retroviral vectors, liposomes, polymeric particles and metallic nanoparticles have been used as carriers to deliver siRNA within cancer-site. However, protecting siRNA from serum degradation and cytoplasmic delivery are two major issues. Also, in most cases, a mere siRNA mediated oncogene knockdown does not have significant impact on the cancer cell apoptosis since the cells adapt to another effector survival pathway. To overcome these challenges and understand the adopted effector downstream mechanism post oncogene knockdown, we hierarchically created a well-defined 200nm tri-block nanocomplex with each sublayer contributing to a definite function. The nanocomplex comprises of an enzymatically cleavable protein (gelatin) nanoparticle encapsulated with a TKI (gefitinib) and surface functionalized with an antibody (Cetuximab)-siRNA (Kras G12C) conjugate. Detailed characterization revealed each nanoparticle of the tri-block nanocomplex comprised of ~400 antibodies and ~800 siRNA. We protected 14KDa siRNA within 150KDa cetuximab and sandwiched it between antibody and gelatin nanoparticle to protect from serum degradation, confirmed using SDS-PAGE. To investigate cellular activity, we incubated the nanocomplex in drug resistant Kras G12C mutant NSCLC (H23 cells). The nanocomplex, when delivered to cytoplasm of the drug resistant H23 cells for oncogene knockdown, sensitized the affected cells to the co-delivered TKI. Knockdown of the oncogene was confirmed by monitoring the PI3K and MAPK downstream protein expression levels. Western blot results indicated abrogation of activated PI3K and MAPK pathway proteins. In vitro assays revealed 95% toxicity for the nanocomplex containing 5µM gefitinib as against 0-10% toxicity for 5µM stand-alone gefitinib. rtPCR showed downregulation of DUSP6, a known effect for H23 cells with knocked down oncogene. Flow cytometry results showed 2 fold higher internalization of the nanocomplex compared to transfected siRNA. However, in the absence of TKI, the nanocomplex showed no toxicity suggesting the cells adapt to a parallel effector pathway for survival, although phosphorylated Mek, Erk and Akt were downregulated leading us to investigate a possible survival mechanism. We hypothesized that the downstream signaling is governed by Gab1 assisted pathway. In H23 cells, activated ERK results in phosphorylation of Gab1 on serine and threonine residues and forms Gab1-p85 PI3K complex that are adjacent to p85 PI3K binding sites. Knocking down the oncogene dephosphorylated Erk, and negated the complex formation. This further cascaded in tyrosine phosphorylation at Tyr627 domain of Gab1, which is known to associate with downstream signaling proteins. Western blot results indicated abrogation of activated PI3K and MAPK pathway proteins. In vitro assays revealed 95% toxicity for the nanocomplex containing 5µM gefitinib as against 0-10% toxicity for 5µM stand-alone gefitinib. rtPCR showed downregulation of DUSP6, a known effect for H23 cells with knocked down oncogene. Flow cytometry results showed 2 fold higher internalization of the nanocomplex compared to transfected siRNA. However, in the absence of TKI, the nanocomplex showed no toxicity suggesting the cells adapt to a parallel effector pathway for survival, although phosphorylated Mek, Erk and Akt were downregulated leading us to investigate a possible survival mechanism. We hypothesized that the downstream signaling is governed by Gab1 assisted pathway. In H23 cells, activated ERK results in phosphorylation of Gab1 on serine and threonine residues and forms Gab1-p85 PI3K complex that are adjacent to p85 PI3K binding sites. Knocking down the oncogene dephosphorylated Erk, and negated the complex formation. This further cascaded in tyrosine phosphorylation at Tyr627 domain of Gab1, which is known to associate with downstream of EGFR but upstream of Ras, to regulate EGFR signaling through several positive feedback loops. We found that TKI binds to this specific phosphotyrosine domain of Gab1, the domain that is responsible for Gab1-Egfr association. In the absence of a TKI, the feedback loop mediated via Gab1 provides a route for survival but is sensitized by abrogation of Gab1-Egfr complex formation post oncogene knockdown when exposed to a TKI. The outcome of this study