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

Srikar Raman, Dhananjay Suresh, Ajit Zambre, Kristen H. Taylor, Raghuraman Kannan *University of Missouri, Columbia, MO*

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 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

provides a potential solution for treating patients harboring KRAS mutation.

miR-342-3p regulates MYC transcriptional activity via direct repression of E2F1 in human lung cancer

Mei Chee Tai,[1] Taisuke Kajino,[1] Masahiro Nakatochi,[2] Chinatsu Arima,[1] Yukako Shimada,[1] Motoshi Suzuki,[1] Hiroyuki Miyoshi,[3] Yasushi Yatabe,[4] Kiyoshi Yanagisawa,[1] Takashi Takahashi[1] *[1]Division of Molecular Carcinogenesis, Center for Neurological Diseases and Cancer, Nagoya University, Nagoya, Japan, [2]Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, Nagoya, Japan, [3]Subteam for Manipulation of Cell Fate, BioResource Center, RIKEN, Tsukuba, Japan, [4]Department of Pathology and Molecular Diagnostics, Aichi Cancer Center Hospital, Nagoya, Japan*

Accumulating evidence indicates that altered miRNA expression is crucially involved in lung cancer development, though scant information is available regarding how MYC, an archetypical oncogene, is regulated by miRNAs, especially via a mechanism involving MYC cofactors. Although various oncogenes have thus far been identified to be altered in various types of lung cancer, MYC is among the most frequently amplified and overexpressed. The MYC gene encodes a transcription factor that regulates a wide variety of genes involved in control of cell growth, proliferation, and apoptotic cell death. The transcriptional activity of MYC is tightly controlled for proper transcriptional regulation through various mechanisms, which include MYC expression itself at both transcriptional and posttranscriptional levels, as well as its interaction with cofactors that functionally cooperate with MYC. Unfortunately, very little is known thus far about how MYC is regulated by miRNAs in lung cancer cells, especially via the latter mechanism involving MYC cofactors.

In this study, we attempted to identify miRNAs involved in regulation of MYC transcriptional activity in lung cancer. To this end, we utilized an integrative approach with combinatorial usage of miRNA and mRNA expression profile datasets of patient tumor tissues, as well as those of MYC-inducible cell lines in vitro. Our results allowed us to identify multiple miRNAs reported as either directly downstream or upstream of MYC, supporting the robustness of our strategy. The former examples included the miR-17-92 cluster, miR-22, miR-26a, miR-30a-3p, and miR-30e-3p, all of which were previously shown to be under MYC-mediated transcriptional regulation, while the latter instances were comprised of let-7, miR-34a and miR-24, which have been reported to directly repress MYC expression via binding to a target site at the 3'UTR of MYC. Intriguingly, our integrative approach also led us to identify miR-342-3p, which we found to be a miRNA indirectly regulating MYC activity via direct inhibition of E2F1, a MYC-cooperating transcription factor. Furthermore, miR-342-3p module activity, which we defined as a gene set reflecting the experimentally substantiated influence of miR-342-3p on mRNA expression, was found to be inversely correlated with MYC activity reflected by MYC module activity in 3 independent datasets of lung adenocarcinoma patients. Our present findings also clearly demonstrate that miR-342-3p plays important roles to inhibit cell cycle progression and proliferation in lung adenocarcinoma cell lines.

Taken together, our integrative approach appears to be useful to elucidate inter-regulatory relationships between miRNAs and protein coding genes of interest, even those present in patient tumor tissues, which remains a challenge to better understand the pathogenesis of this devastating disease.

Loss of immunoproteasome driven by EMT is associated with immune evasion and poor prognosis in non-small cell lung cancer

Satyendra C. Tripathi,[1] Haley L. Peters,[1] Ayumu Taguchi,[1] Hiroyuki Katayama,[1] Hong Wang,[1] Amin Momin,[1] Mohit K. Jolly,[2] Muge Celiktas,[1] Jaime Rodriguez,[1] Hui Liu,[1] Carmen Behrens,[1] Ignacio I. Wistuba,[1] Herbert Levine,[2] Jeffrey J. Molldrem,[1] Samir M. Hanash,[1] Edwin J. Ostrin[1] *[1]The University of Texas MD Anderson Cancer Center, Houston, TX, [2]Rice University, Houston, TX*

Immunoproteasome are a specialized form of multisubunit complexes called proteasome that degrade intracellular proteins through the ubiquitin-proteasome pathway. It can generate peptides with high specificity for binding onto MHC class I molecules, hence a suitable candidate for CD8+ T cell mediated cytotoxic responses. The expression of the immunoproteasome and its impact on antigen presentation in tumors of epithelial origin is not well established. We have investigated the constitutive and induced expression patterns of immunoproteasome subunits in non-small cell lung cancer (NSCLC) and their consequence on antigen presentation. We also assessed the impact of immunoproteasome expression on survival in early stage NSCLC.