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

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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

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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.
Significantly reduced expression of immunoproteasome components and their regulators was observed to be associated with epithelial to mesenchymal transition in 42 NSCLC cell lines using proteomic profiling and microarray analysis. A highly variable immunoproteasome expression was also observed among NSCLC tissues. Immunohistochemistry data revealed loss of immunoproteasome subunit is significantly correlated with expression of CDH2 and concomitant loss of CDH1 in NSCLC tumors. Loss of immunoproteasome subunits was also significantly associated with advanced stage (p = 0.014), recurrence (p = 0.002) and metastasis (p < 0.01) in NSCLC patients. A significantly reduced antigen presentation was observed in mesenchymal cell lines compared to epithelial cells. Using mild acid elution, only 50-60 HLA class I bound peptides were identified in mesenchymal cell lines compared to 400-500 peptides in epithelial counterparts. IFNγ as well as 5-aza-2’-deoxycytidine treatment was able to revive immunoproteasome expression and hence restored repertoire of HLA class I bound peptides in deficient mesenchymal cells. Induced expression of immunoproteasome and hence HLA class I bound peptides also lead to significantly enhanced CD8+ T cell mediated cytotoxicity (p < 0.01) in mesenchymal cells compared to non-induced controls. Our findings point towards a mechanism of immune evasion of cells with a mesenchymal phenotype and strategies to overcome their immune evasion through induction of the immunoproteasome or targeting the limited repertoire of peptides presented in common by these cell types.

NRG-LU001: A phase II trial investigating metformin as a chemo-radio-sensitizer in locally advanced non-small cell lung cancer (NSCLC)

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Several lines of evidence indicated that the anti-diabetic agent metformin may have significant anti-tumor activity. This is an economical and effective anti-diabetic agent used by more than 120 million patients worldwide and is well-tolerated by non-diabetics too. Metformin is believed to mediate anti-tumor action through blockade of mitochondrial OxPhos complex I and induction of a stage of mild metabolic stress that activates the AMP-activated kinase pathway (AMPK), an enzyme with tumor suppressor activity.

We showed that AMPK is a sensor of both metabolic and genotoxic stress that mediates cell cycle arrest and tumor suppression. Our pre-clinical translational and retrospective clinical studies suggested that targeting metabolism in NSCLC with metformin enhances NSCLC radio-sensitivity and could improve outcomes in locally advanced NSCLC.

NRG-LU001 (NCT02186847) is an NCI-CTEP funded trial that opened to accrual in August 2014. It is designed to examine specifically whether metformin could radiochemo-sensitize NSCLC in locally advanced stage III (A and B) patients. Its primary outcome is 1 year progression free survival (PFS) and secondary outcomes include: overall survival (OAS); time to local-regional progression (LRP); time to distant metastasis (DM); Chemo-RT toxicity; biospecimen collection for biomarker analysis. This will include: circulating serum and blood cell biomarkers of metformin activity and tumor response and tumor biomarkers such as tumor histology, TP53, LKB1 and K-Ras mutation status.

NRG-LU001 target accrual is 168 patients with 1:1 randomization to either chemo-radiotherapy (CRT) alone vs CRT and metformin of 2000mg daily given only during cytotoxic therapy. This includes concurrent CRT for 6 weeks followed by 6 weeks of consolidation chemotherapy treatment. Chemotherapy, in both the concurrent and the consolidate phase of this study is the carboplatin-paclitaxel doublet, while patients receive standard chest RT of 60 Gy in 30 fractions. Sample size calculations are based on observed 1-year PFS of 50% (RTOG-0617 data) and an expected improvement of 15% with metformin. With 152 analyzable patients this study will have 85% power to detect this improvement. The number is increased to 168 to allow 10% rate of ineligibility.

LU001 is currently open in 52 centers across North America. This is one of the first clinical trials investigating the potential of metabolism modulating agents to enhance chemo-RT responses in locally advanced NSCLC. It is expected to provide initial efficacy results for this agent and assist in the investigation and development of circulating and tumor biomarkers of metformin action and sensitivity. If positive this study will allow us to design rationally future phase III studies to examine definitively the role of metformin in this setting.