Nonclassical activation of gli1 as a therapeutic target for squamous cell lung cancer

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Background: The Hedgehog (Hh) signaling pathway is critical for embryonic development and its deregulation is implicated in a number of tumor types. The role of the Hh signaling pathway, however, in the initiation and growth of non-small cell lung cancer is largely unknown. Here, we investigate the role of the Hh pathway transcription factor, GLI1, in lung squamous cell carcinoma (SCC) and as a potential therapeutic target for treatment of lung SCC.

Methods: GLI1 expression in human SCC cell lines was evaluated by quantitative PCR and Western Blot. siRNA and shRNA of GLI1 in these cell lines were utilized in vitro and in vivo to test the requirement of GLI1 in tumor growth. Small molecule modulators of GLI1 were tested for their therapeutic potential.

Results: GLI1 mRNA expression was significantly elevated in lung SCC compared to normal lung and lung adenocarcinoma patient specimens in several human genomic databases. Importantly, overexpression of GLI1 was correlated with poor overall survival in lung cancer patients. siRNA-mediated knock down of GLI1 in SCC cell lines decreased the expression of GLI1 target genes and caused a significant reduction in colony formation. Stable knock down of GLI1 in SCC cell lines caused a significant reduction in growth of xenograft tumors indicating the critical role of GLI1 in lung SCC progression. Inhibition or activation of SMO, an upstream component of Hh pathway, did not alter GLI1 expression level in lung SCC cell lines. However, inhibition of PI3K/AKT and MAPK signaling pathways down-regulated GLI1 expression, suggesting that GLI1 expression is dependent on PI3K/AKT and MAPK pathway activity rather than Hh ligand. Small molecule inhibition of PI3K/mTOR pathway or GLI1 significantly reduced GLI1 expression, proliferation, and clonogenicity in SCC cell lines. Combinatorial inhibition of PI3K and GLI1 by BKM120 and arsenic trioxide (ATO), respectively, significantly abrogated the in vivo growth SCC tumors in mice and correlated with decreased tumor GLI1 expression.

Conclusion: Our findings demonstrate that GLI1 is essential for lung SCC tumor progression. Furthermore, GLI1 expression in SCC is independent of Hh pathway ligand action and dependent on MAPK and PI3K pathway activity. Direct inhibition of GLI1 by repurposing ATO in combination with a PI3K inhibitor may represent a novel therapeutic strategy for lung SCC.

Validation of L-Myc as a viable therapeutic target in small cell lung cancer

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Background and Hypothesis: The paucity of molecular targets for small cell lung cancer (SCLC) chemoprevention and therapy is largely due to the poor understanding of SCLC progression beyond the role of RB and P53 mutations crucial for tumor initiation. Amplification of the Myc family of oncopgenes is one of the most frequent alterations in human SCLC genomes. However, the concept of inhibiting these factors to intervene in SCLC progression, despite its clear value as a targeted therapy, has not been formally tested in the autochthonous model. We tested the hypothesis that L-Myc is a key determinant of SCLC and its pathway as a viable target for therapeutics and chemoprevention.

Methods and Results: Using comparative gene expression analysis of pre-cancerous cells (preSC) and tumor cells, both derived from the genetically engineered mouse model (GEMM), we identified a gene set specific to SCLC tumorigenic progression and found that L-Myc is the most up-regulated gene in the mouse model. Retroviral overexpression of L-Myc, mimicking the gene amplification, was sufficient to cause the tumorigenic progression of the L-Myc-expressing preSC in culture or allograft experiment, while CRISPR-mediated knockout of L-Myc blocked the long-term growth of SCLC cells in culture. Comparison of L-Myc-preSC with control (non-transformed) preSC revealed a specific gene signature, and the pathway analysis of the signature indicated significant activation of several molecular pathways, including epithelium-to-mesenchyme transition, downstream of L-Myc during tumor progression. More significantly, conditional deletion of L-Myc in the GEMM dramatically reduced tumor burden in a
dose-dependent manner and significantly extended survival of the mice.

**Conclusions:** These results provide comprehensive evidence for the oncogenic functions of L-Myc and further support the concept of targeting the gene and its related molecular pathways to intervene in SCLC. Additionally, the new approaches demonstrated in this study will facilitate functional analysis of numerous candidate genes, increasing the likelihood of determining cancer-relevant genes and pathways.

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**E2F8 and its target genes as novel therapeutic targets for lung cancer**

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Lung cancer remains a major cause of cancer mortality in the world. There is a significant need to develop new strategies that provide effective treatment for lung cancer. Current study reports that targeting oncogenic transcription factors could be a potential treatment method for lung cancer. The E2F transcription factor family members have been shown to be involved in cancer development. The E2F members have been divided into transcription activators (E2F1-E2F3) and repressors (E2F4-E2F8). E2F8 with E2F7 has been known to play an important physiologic role in embryonic development and cell cycle regulation by repressing E2F1. We found that E2F8, an E2F transcription factor family member, is overexpressed in lung cancer cell lines and tumors from lung cancer patients compared with normal lung cells and tissues, as determined by immunoblotting or immunofluorescence staining in human lung cancer cells and tissues from lung cancer patients. Kaplan-Meier analysis of data from a public database showed that aberrantly overexpressed E2F8 in patients with lung cancer is associated with worse prognosis. Depletion of E2F8 inhibited cell proliferation, colony formation, invasion and tumor growth in vitro and in vivo, while growth of normal cells was not affected by the loss of E2F8. In addition, depletion of E2F8 induced substantial DNA damage in cancer cells but not in normal cells. Moreover, targeting E2F8 using its specific siRNAs and morpholino-modified antisense dramatically suppressed tumor growth in vivo studies using mouse models, including s.c. xenograft in nude mice, syngeneic mouse lung cancer model, and a transgenic lung cancer mouse model. To identify genes regulated by E2F8, we performed microarray analyses using human lung cancer cell lines (NCI-H1975, H441, and H520) and Affymetrix Human Genome Arrays. Bioinformatical analyses revealed that knockdown of E2F8 deregulated gene sets involved in regulation of transcription, cancer progression, chromatin organization, regulation of immune system, glutamate receptor signaling, and cell surface receptor signaling. Further analysis of E2F8 binding motif using chromatin immunoprecipitation (ChIP) assays combined with sequencing (ChIP-Seq) method, we identified genome-wide distribution of 204 E2F8 binding sites. From the microarray analysis and ChIP-Seq assay, we identified the UHRF1 (ubiquitin-like PHD and RING domain-containing 1), critical for DNA replication of cancer cells, as one of the E2F8 target genes. In conclusion, we report that E2F8 is overexpressed in lung cancer and is required for the growth of lung cancer cells. The E2F8 knockdown significantly perturbed genes involved in the DNA replication pathway in cancer cells. These findings provide evidence that E2F8 is a novel therapeutic target for lung cancer treatment.

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**Rare but poor prognosis of TERT promoter mutation in non-small cell lung cancer patients**

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The mutation in the promoter region of telomerase reverse transcriptase (TERT) and telomere length have been focused in various cancers. In present study, the frequency and clinical characteristics of TERT promoter mutation and telomere length were studied in non-small cell lung cancers (NSCLC). TERT promoter mutation and telomere length were analyzed in 188 patients by using sequencing and real-time PCR, respectively. The TERT promoter mutation rate was 2.2% (4/188) of NSCLC and it was associated with regional lymph node invasion (p < 0.001) and poor differentiation (p = 0.060). Telomere length was not associated with TERT promoter mutation and it divided into high and low groups by median value (3.04). Telomere length was shorter in males (p = 0.058) and smokers (p = 0.008). Survival analyses showed a poor prognosis of NSCLC with TERT promoter mutation (p < 0.001). Multivariate survival analyses demonstrated that TERT promoter mutation was associated with poor overall survival (p = 0.045). These data demonstrated TERT promoter mutation was not frequent in NSCLC, however, it might have a potential value for prognostic factor in NSCLC.