Urokinase Plasminogen Activator Expression is Regulated by p53 Harboring the Lung Cancer-Specific Mutation V157F

Julie A. Barta, MD1; Kristen Pauley2; Steven B. McMahon, PhD2

1Korman Respiratory Institute, Division of Pulmonary and Critical Care Medicine; 2Department of Biochemistry and Molecular Biology; Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia

OBJECTIVES
- To define the mutant p53-regulated transcriptome of lung cancer cells with alterations at V157 and R158 in the p53 tumor suppressor.
- To determine the biological effects of lung-enriched p53 mutations in lung cancer cells.

BACKGROUND
- Missense mutations in the tumor suppressor gene TP53 occur in 54% of lung adenocarcinomas and 89% of squamous cell lung cancers. The most common mutations in p53 render the protein not only unable to perform its tumor suppressive function but frequently also lead to a gain of oncogenic function (GOF).
- We and others have shown that lung tumors are enriched for missense mutations in p53 at codons 157 and 158, a distinct lung “cluster.”
- The gene PLAU encodes urokinase-type plasminogen activator (uPA), a key regulator of extracellular matrix degradation via the plasminogen activation system, uPA is highly expressed in several solid tumor types and is associated with poor clinical prognosis.
- We hypothesize that p53 mutations at V157 and R158 directly regulate gain of oncogenic function through changes in target gene expression, specifically in lung cancer.

METHODS
- The human lung cancer cell lines A549 (wt p53), H2087 (V157F mut p53), H441 (R158L mut p53), and H2110 (R158P mut p53) were obtained from ATCC and were cultured in standard tissue culture conditions. To determine the effects of transient p53 knockdown, cells were transfected with 25 nM sip53. At 72 hours post-transfection, cells were lysed and secreted proteins were harvested for analysis.
- RNA-sequence analysis was carried out by the Wistar Institute Genomics and Bioinformatics Core Facilities. Cell sequencing reads were aligned to human genome hg19 using Bowtie2, and differentially expressed genes were identified using DESeq2 method. Core Facilities. Cell sequencing reads were aligned to human genome hg19 using Bowtie2, and differentially expressed genes were identified using DESeq2 method.
- Western blotting was performed to detect protein expression using antibodies against p53 (DO-1) and uPA.
- Cell migration was measured using a standard wound healing assay. A549 and H2087 cells were seeded in 24-well plates at 100,000 cells/ well and 200,000 cells/well, respectively, and were fully confluent at 48 hours when a scratch was made. Images were taken at regular intervals and scratch diameter was calculated as percentage of 100% scratch closure.

RESULTS
- To our knowledge, GOF phenotypes in lung cancer cells harboring V157F mutp53 have not been characterized.
- We have shown here that mutp53 represses uPA expression in human lung cancer cells with endogenous V157F mutp53, and depletion of p53 in these cells leads to an increase in migration.
- These findings prompt a re-examination of the signaling pathways associated with uPA. Further study is needed to elucidate the mechanism of the p53-dependent alterations in uPA expression and to establish additional biological effects of V157F mutp53.

CONCLUSIONS
- V157F, R158L, and R158P mutant p53 regulate distinct groups of genes (Figure 1) with common biological functions (Table 1) in lung cancer cells harboring these lung-enriched mutations. Notably, mutant p53 represses genes associated with functions including cell viability, migration, and invasion.
- With p53 depletion, protein (Figure 2A) and mRNA (Figure 2B) expression of the uPA pro-enzyme pro-uPA and its activated B- and A-chains increased in the V157F mutant p53 lung cancer cell line H2087. Secreted uPA also increased as measured in conditioned media from H2087 cells (Figure 2A).
- Cell migration is increased by V157F mutant p53-depleted H2087 cells compared with mutant p53-expressing H2087 cells, while A549 cells (wt p53) showed no p53-dependent difference in migration rate (Figure 3).

REFERENCES
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