Assessing gene expression in lung subcompartments utilizing in situ RNA preservation.

The mechanisms of toxicant-mediated lung injury and repair are influenced by the considerable spatial heterogeneity that exists within the conducting airways of the lungs. As a result of this heterogeneity, significant differences and similarities in gene expression are observed throughout lung subcompartments. RNA-based technologies such as real-time reverse transcription polymerase chain reaction (real-time RT-PCR) and cDNA microarray analysis of gene expression provide valuable clues to understanding the mechanisms of toxicant-induced injury. Isolating RNA from lung subcompartments has previously involved considerable time and labor-intensive processes that limit the number of animals that could be processed in a day. The aim of this study was to determine if intact, high-quality RNA could be preserved in situ over a period of time to delay the need to immediately perform site-specific lung subcompartment microdissections and RNA isolations. Two hours after 1-nitronaphthalene treatment, rat lungs were inflated with and stored in RNA preservation solution and stored at 4 degrees C for 7 days. RNA was isolated from the lung subcompartments isolated by microdissection. After 7 days of storage, the RNA was intact, of high quality, and could be used for real-time RT-PCR to examine heterogeneous gene expression in the lung subcompartments. In summary, this simplified technique of in situ RNA preservation and site-specific lung subcompartment microdissection allows the isolation of intact, high-quality RNA that may be used for gene expression analysis.
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Published on UAB School of Public Health (http://www.soph.uab.edu)

used with molecular RNA-based technologies that will significantly accelerate our understanding of pulmonary injury and repair mechanisms.

DOI

10.1093/toxsci/kfh002

Alternate Journal

Toxicol. Sci.

PubMed ID

14600286

Grant List

ES00628 / ES / NIEHS NIH HHS / United States
ES04311 / ES / NIEHS NIH HHS / United States
ES06700 / ES / NIEHS NIH HHS / United States
HL7013 / HL / NHLBI NIH HHS / United States