Assessing the quality of human pediatric lung organ sample preparation by the Human Tissue Core for the Molecular Atlas of Lung Development (LungMAP) program.

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Rationale: Access to developing human lung is a barrier to advancing medical treatments for neonatal and pediatric lung disease. The NHLBI established the LungMAP program to create a better understanding of human lung development. Data generated will rapidly be made available to the scientific community to be used for the basis of future studies. A centralized Human Tissue Core (HTC; funded by U01HL122700), at the University of Rochester and in collaboration with Seattle Children's Hospital, will provide human lung tissue and cells to four research centers that employ cutting-edge technologies to generate high-quality big-data sets. Methods include lipidomics, metabolomics, transcriptomics, epigenetics, flow cytometry and fluorescence microscopy. We will present data illustrating the quality and diversity of sample isolated from 10 donor lungs ranging from 1-day-old neonates up to eight-year-old children.

Methods and results: Given that a wide variety of techniques will be used in the research centers, the HTC is working with Organ Procurement Organizations (OPOs) to procure tissue with warm and cold ischemic times of less than 1 and 12 hours, respectively, in order to provide samples with the transplant quality integrity. Our hypothesis is that by using OPOs, the HTC will be able to provide high quality tissue to LungMAP research centers in a timely manner. Our efforts focus on processing the lung for a variety of downstream applications. We have optimized computed tomography (CT) scanning methods to assess gross tissue structure. Furthermore, we have developed cryopreservation and fixation methods for inflating lung. Annotated, histological stained, whole slide images will be reviewed by experienced pediatric pathologists and by the research community accessing the www.LungMAP.net to assess the quality, structure and integrity of tissue. We tested various mechanical and enzymatic combinations (n=9) to dissociate tissue, increasing viability and yield of enriched cell populations phenotyped by flow cytometry with specific antibodies (n=6). Methods for sorting epithelial, endothelial, lymphatic, immune and mesenchymal cells using Fluorescent Activated Cell Sorting are being optimized. We demonstrated successful culture of these sorted cell populations from cryopreserved dissociated lung tissue cells. Furthermore, mesenchymal stromal cells have been differentiated (n=6), taking on culture-condition dependent characteristics of adipocytes, chondrocytes, and osteoblasts.

Conclusions: We have established a system for the efficient procurement, processing, and quality assessment of pediatric lung organ donations. The specimens obtained from this workflow will be used by the LungMAP research centers to provide comprehensive datasets of human lung development to the lung biology community.