Web Material

Tracing Lung Cancer Risk Factors Through Mutational Signatures in Never Smokers: the Sherlock-*Lung* Study

Maria Teresa Landi, Naoise C. Synnott, Jennifer Rosenbaum, Tongwu Zhang, Bin Zhu, Jianxin Shi, Wei Zhao, Michael Kebede, Jian Sang, Jiyeon Choi, Laura Mendoza, Marwil Pacheco, Belynda Hicks, Neil E. Caporaso, Mustapha Abubakar, Dmitry A. Gordenin, David C. Wedge, Ludmil B. Alexandrov, Nathaniel Rothman, Qing Lan, Montserrat Garcia-Closas, and Stephen J. Chanock.

Table of Contents

WEB FIGURE 1: SHERLOCK-*LUNG* OPTIMAL SAMPLE REQUIREMENTS 2
WEB APPENDIX 1: SUMMARY OF LABORATORY SAMPLE PROCESSING 3

Sherlock lung

Tracing Lung Cancer Mutational Processes in Never Smokers

National Cancer Institute (NCI) Division of Cancer Epidemiology and Genetics (DCEG)

CALL FOR BIOSPECIMENS AND DATA

Goals

To investigate the etiology and progression of lung cancer in never smokers from the general population as well as from populations with substantial levels of special exposures through (1) an integrative genomic analysis and (2) pathology, clinical, radiological, and epidemiologic assessment

Major aims:

1. To identify exogenous exposures and endogenous processes involved in lung tumorigenesis in never smokers
2. To develop an integrated molecular, histologic & radiologic classification of lung cancer in never smokers

Target Study Population

Never smokers diagnosed with lung cancer from the general population (~1500 cases) and from populations with special exposures, e.g., smoky coal, radon, asbestos, indoor and outdoor air pollution, previous chronic lung diseases (~500 cases)

Materials Sought (collected before therapy)

Minimum required materials:

- Fresh frozen tumor tissue (preferably from surgery, otherwise ≥2 biopsies); paired germline material (normal lung tissue and/or blood); and data

Additional specifications and preferred materials:

- Fresh frozen tumor tissue (T) plus source of germline (G) DNA, or DNA and RNA extracted from T and G

- Paired fresh frozen normal tissue (N) is highly desirable (multiple samples preferred), or DNA and RNA extracted from N
- Tissue characteristics: T (>60% tumor cells, <20% necrosis), N (no tumor cells); optimal 40mg x 2, minimum 40mg
- DNA: optimal ≥8ug, minimum ≥4ug; RNA optimal ≥5ug (RIN≥7), minimum ≥2ug (RIN≥6)

- Blood (B) 500uL whole blood or buffy coat, or DNA extracted from B
- H&E slides (to be scanned and returned) or H&E images from every tissue block for every tumor
- Multiple FFPE blocks (at least one from tumor center and one from tumor periphery), or sections from FFPE blocks

- Blocks: to be sectioned and remaining material returned
- Sections: (T)) optimal 10 sections x 10um, min 5 sections x 10um; (N) optimal 20 sections x 10um, min 10 sections x 10 um

- Plasma (P) optimal ≥7 ml, minimum ≥2 ml
- De-identified pathology reports or data extracted from pathology reports
- De-identified radiology reports or data extracted from radiology reports
- All available de-identified chest CT, PET, and x-ray images (if CT and/or PET available, x-ray not required)

For special exposures, or population, relaxation of required elements may be considered based on information provided by PIs

Data Sought

- Epidemiological data (e.g. demographics, BMI, smoking, passive smoking, history of lung disease, personal/family history of cancer, medical history, chronic meds/NSAID, occupational and environmental exposures, detailed residential history)
- Clinical data (e.g. histology, survival, stage, grade, local recurrences, metastases, multiple primaries, treatment categories e.g. chemo, radiation, immunotherapy, surgery, hormonal, biological, palliative)

Materials Returned

Will return upon request: H&E slides from each tumor block; remaining DNA, RNA, tissue samples; BAM/VCF files of sequencing data after final QC assessment of genomic characterization

Contact

Scientific questions: Maria Teresa Landi, M.D., Ph.D. landim@mail.nih.gov 240-276-7236

General questions: sherlocklung@nih.gov

Web Figure 1: Sherlock-*Lung* optimal sample requirements

Web Appendix 1: Summary of Laboratory Sample Processing

Frozen tissue specimens were received and processed by the chosen laboratory. A 5μm section of tissue was cut from the provided block for creation of an H&E slide for pathology review. Specimens passing inclusion criteria (tumor/normal cellularity) were forwarded for total nucleic acid extraction. A two-column approach was utilized for extraction of DNA and RNA from approximately 10-40 mg of tissue, depending on tissue availability. Tissue was homogenized using Qiagen TissueLyser II (Qiagen, Germantown, MD). Dual DNA and RNA Extraction was performed utilizing the AllPrep™ DNA/RNA Mini Kit (Qiagen, Germantown, MD) for DNA and *mir*Vana™ miRNA Isolation Kit (Applied Biosystems, Foster City, CA) for total RNA and small RNA.

Purified nucleic acids were quantified and quality checked post-extraction. RNA was tested utilizing the Agilent 2100 Bioanalyzer with the RNA 6000 NanoChip (Agilent, Santa Clara, CA). DNA was quantified with the Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher, Waltham, MA). DNA molecular weight was evaluated by E-Gel™ 48 Agarose Gels, 1% (ThermoFisher, Waltham, MA). Tumor and normal tissue DNA samples were identity matched via SNP analysis using the MassARRAY™ system and iPLEX™ chemistry (Sequenom, San Diego, CA), across 50 loci.