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Feasibility of lung cancer RNA acquisition from a single transbronchial or transthoracic needle pass (FASTT trial)

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ABSTRACT

Introduction: RNA isolation from tumor tissue is used for biomarker analyses and validation. Limited diagnostic material from small volume biopsies combined with an increasing demand for standard histologic, molecular characterization, and next generation sequencing applications often leads to limited material for research. We sought to evaluate small volume sampling of lung cancer tissue collected from a single needle pass during a diagnostic procedure and determine if it can provide RNA of acceptable quantity and quality.

Methods: We enrolled 140 patients with probable primary bronchogenic carcinoma and collected RNA from a dedicated FNA aspiration. Total RNA (ηg), RNA integrity number (RIN), and %Mass in base pairs were evaluated from each patient sample. A customized nanoString nCounter® 95-gene panel was used to profile the expression patterns of feature NSCLC genes. We compared gene expression patterns that distinguish lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) in our cohort with a corresponding Cancer Genome Atlas (TCGA) NSCLC datasets.

Results: Of the 149 patients consented. RNA-extraction was performed in 101 eligible patients. A satisfactory total RNA mass and RIN was quantified for all samples with a similar distribution among cellular subtypes. Mean %-Mass over 300 base pairs was noted for all specimens and 96% of samples met criteria to perform genetic evaluation with our commercialized gene expression assay. The FNA-derived transcriptomic results showed excellent consistency with the TCGA counterparts, and the differential expression pattern of LUAD vs LUSC subtypes were highly similar.

Discussion: In this study, RNA retrieval from a single-pass FNA regardless of procedural approach showed equivalence and suitability for gene expression assessments. RNA extraction from small volume samples has the potential to provide valuable material for genetic profiling.

1. Introduction

The biology of non-small cell lung (NSCLC) cancer is complex with considerable variation in the clinical behavior and treatment of tumors with specific genetic mutations, rearrangements, high/low PD-L1 expression, and variable histologies. Many tumor markers for testing at diagnosis are required to assure that all patients with actionable targets are treated with the most efficacious drug. Diagnostic specimens from cytological material need to classify histology, standard mutations, and PDL-1 expression, irrespective of how a patient's biopsy is obtained.

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The majority of lung cancer cases occur with advanced or metastatic disease. These patients generally undergo the least invasive diagnostic method which in turn limits the number of specimens. Maximizing the available tissue obtained through small volume fine needle aspirates (FNA) from bronchoscopy or trans-thoracic applications is paramount. Published guidelines now underscore the importance of identifying PDL-1 expression, EGFR mutations, ALK and ROS-1 rearrangements, and histological classification [1]. In clinical practice, there is more attention placed on the triaging of specimens for these molecular tests, with decreasing importance on differentiating NSCLC further into histologic subtypes. Documenting an adenocarcinoma histology often serves as the gatekeeper to further testing for molecular targets. However, this strategy is likely to miss undifferentiated variants of adenocarcinoma or other histologic entities that may harbor driver mutations. Rekhtman commented on the important balance of driver mutation identification for all NSCLC and tissue resource utilization [2].

Limited diagnostic material from small volume biopsies combined with an increasing demand for standard histologic, molecular characterization, and next generation sequencing applications often leads to consumption of all the available material. Often there is insufficient material to complete these points of characterization, and limited ability to acquire samples or bank specimens for future research. As precision medicine expands so will the challenge of research and other biomarker analysis from sparse diagnostic tissue. Analytical platforms better optimized for small volume biopsies will be required [1–3].

Ribonucleic acid (RNA) analysis is common with many preexisting platforms for research biomarker analysis. It has also made it into standard clinical practice with examples like Oncotype DX® which is an RNA based genomic tool that has refashioned the risk assessment and treatment of breast cancer and is incorporated into clinical guidelines [4,5]. Similar RNA based genomic platforms exist for prostate and colon cancer evaluation as well. Although not as routinely employed, the use of RNA based genomic testing to modify treatment strategies for lung cancer is emerging. The Percepta test, an RNA based bronchial genomic classifier that improves the diagnostic performance of bronchoscopy for the detection of lung cancer is evidence of this trend [6].

The ability to utilize RNA as a reliable genomic test hinges on the ability to ascertain RNA of acceptable quality and quantity to perform genetic analysis. An earlier published pilot study demonstrated FNAs of resected tumor could successfully yield the required RNA mass [7]. Other studies pulled archived samples from EBUS-TBNA fine needle aspirates and found it feasible to obtain sufficient quantity and quality RNA for gene expression profiling [7–10]. Patriella et al. successfully extracted RNA from 20 samples of stored FNA material [11]. Schmid-Bindert and colleagues could demonstrate through prospective collection of 106 small volume biopsies that RNA isolation was possible in over 95% of cases [12].

Although minimally invasive proceduralists are evaluating ways to enhance sample acquisition to meet the increasing tissue needs, a precise amount of satisfactory quantity is difficult to define [3]. We sought to evaluate the amount of RNA that can be retrieved from a single FNA through different diagnostic approaches, and its ability to provide clinically relevant and reproducible gene expression utilizing a commercially available NanoString platform.

2. Methods

This study was conducted from 2013 to 2015 at Wake Forest Baptist Health Medical Center and the study protocol was independently reviewed and approved by the institutional review board (study # IRB00020151). All patients enrolled were consented to undergo one additional small volume aspirate for RNA collection prior to the conclusion of their procedure.

2.1. Eligibility

Eligibility criteria included patients that had radiographic evidence for presumed lung cancer or had a previously diagnosed NSCLC with potential recurrence. Patients undergoing FNA of potential NSCLC metastatic lesions were also included (e.g., patients with hepatic metastases). Patient requirements also included age > 18 and the ability to understand and the willingness to sign a written informed consent. Exclusion criteria included patients whose FNA biopsy was unable to provide subtype classification by pathology, consistent with small cell carcinoma, non-malignant etiologies, or returned non-diagnostic.

2.2. Procedure

The majority of small volume FNA diagnostic modalities were obtained by: endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA), conventional trans-bronchial needle aspiration (cTBNA) and trans-thoracic needle biopsy (TTNB). Two additional patients received small volume biopsies in a different manner, as one received an endoscopic ultrasound and the other an ultrasound guided fine needle aspiration. Bronchoscopy cases were performed by pulmonary faculty and fellows using rapid on-site cytology evaluation (ROSE) to help decipher results. TTNB were performed by interventional radiologists with training and experience with CT-guided thoracic procedures.

2.3. Endobronchial ultrasound guided trans-bronchial needle aspiration

Patients scheduled for EBUS-TBNA biopsies had their biopsies performed using an Olympus® BF-Convex Scope XBF-UC160 F-OL8 with a 22 g needle or Pentax® Convex Scope EB-1970UK with a 22 g cytology needle. Sample acquisition was collected in accordance with our standard protocol that includes initial cytology slides for evaluation by ROSE, and 3–5 dedicated needle passes expressed entirely into saline for cell block creation after ROSE indicated a site with diagnostic cellular material. Prior to the conclusion of the procedure one additional needle pass was performed of the primary lesion and all the aspirated material was expressed into 1 ml of RNAlater® and immediately placed on ice.

2.4. Conventional trans-bronchial needle aspiration

Conventional trans-bronchial needle aspiration was performed with the Olympus® BF-1T60 flexible bronchoscope using a 22-gauge cytology needle (Wang Transbronchial Needle, ConMed Endoscopic Technologies, Utica, NY). Like the EBUS-TBNA collection procedure an identical standard protocol was used for the collection of cytological material and prior to the conclusion of the procedure one additional needle pass was obtained of the primary lesion and all of the aspirated material was expressed into 1 ml of RNAlater® and immediately placed on ice. The decision to use conventional bronchoscopy versus EBUS was dependent on operator discretion and influenced by factors such as lymph node or mass size and location on computed tomography and/or fluorodeoxyglucose avidity on positron emission tomography scans.

2.5. CT-guided trans-thoracic needle biopsy

Transthoracic needle biopsies were ordered at the discretion of the treatment team. Patient positioning during the procedure was determined by the radiologist to optimize accessibility to the target lesion. Once the access site was draped and sterilized a 19-gauge coaxial guide needle was advanced under CT fluoroscopic guidance to the target lesion. After imaging clarified safe and adequate positioning of the guide needle, multiple fine needle aspirations (FNA) were collected utilizing capillary technique with 22-gauge Chiba needles. ROSE was immediately available for review and prior to the conclusion of the case

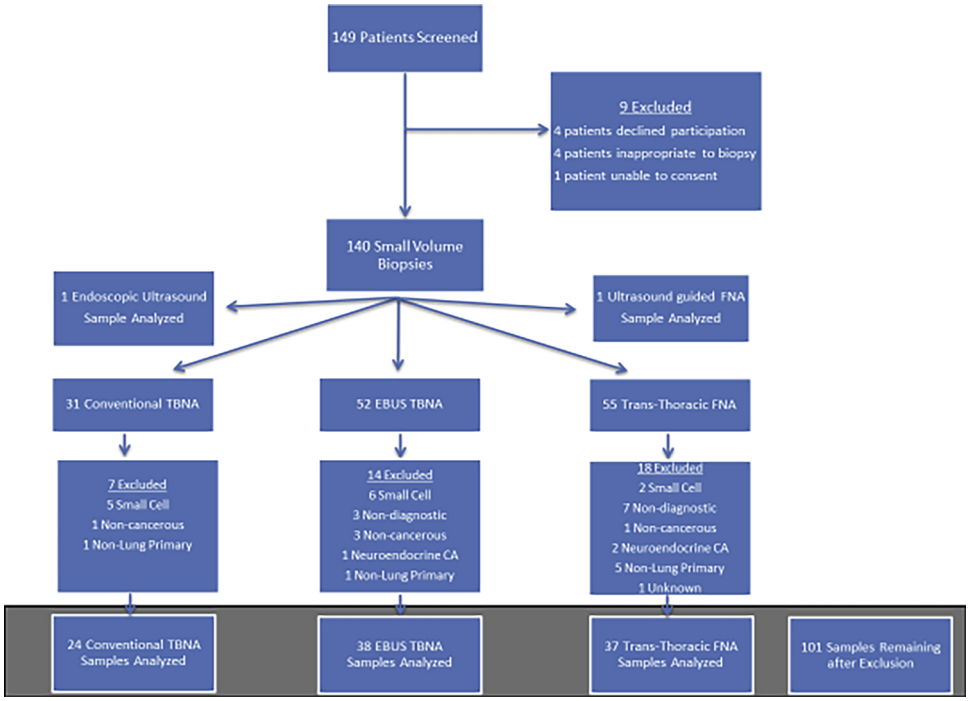

Fig. 1. Diagram of patients included and excluded from the study with breakdown of reason for exclusion.

one additional 22-gauge needle aspiration was performed of the primary lesion and expressed directly into 1 ml of RNAlater® and immediately placed on ice.

All specimens were initially stored in RNAlater® at 4 °C overnight then transferred for storage in a −80 °C until pathologic confirmation was achieved of NSCLC and then the samples were batched and RNA extraction was completed.

2.6. RNA extraction methods

After eligible samples underwent review by a faculty cytologist and confirmed diagnostic, tumor tissue samples collected as the study sample were disrupted under liquid nitrogen and homogenized according to the Qiagen QiaShredder protocol (Qiagen, Valencia, CA). Total RNA (approximately 1–10 µg per sample) was isolated from each tumor sample according to the Qiagen RNeasy Microarray Tissue Mini Kit protocol. Once adequate mass was confirmed using an Eppendorf BioPhotometer, the quality of the RNA was assessed using an Agilent 2100 Bioanalyzer. RNA samples suitable for evaluation were selected using the following criteria: 1) RNA integrity number (RIN) and 2) total mass of RNA 3) %mass greater than 300 base pairs. For gene expression profiling to reliably occur using the NanoString platform standardized RNA recommendations included at least 100 ηg of total RNA submitted and 50% of the RNA mass should be greater than 300bp in length [13].

2.7. Statistical analysis

Statistical analyses were performed using JMP (SAS Institute Inc, Cary, NC). We compared quantitative RNA data by smoking pack years, biopsy modality, and type of malignancy using one-way ANOVA. Results are presented as percentages and means ± standard deviations, unless median is specified. Continuous variables were compared among groups using ANOVA models and, where a signal was present, groups were compared with Wilcoxon Tests. Nominal variables were analyzed using chi-square tests for association. The concordance analysis was performed using single score intraclass correlation with oneway method and F-test (R package “icc”) and Pearson's product-moment correlation with *t*-test (R package “stats”). Linear regression model with F-test (R package “stats”) was used to analyze and remove platform-related effects [14].

2.8. Pathological evaluation

To avoid any bias due to familiarity with the original pathology results, a second research pathologist was enrolled to provide a research pathological read to go along with the clinical pathological read of each specimen. The research pathologist was blinded to the medical record, including clinical and imaging findings, to avoid contact with the original diagnosis. However, the research pathologist did have knowledge that all samples were cytologic specimens of primary lung tumors, and had access to all slides including smears, cell block, and core biopsy material, along with any available immunohistochemical stains.

2.9. Gene expression

In our study, we utilized a 95-gene panel with genes previously identified by microarray expression profiling studies that distinguished LUAD and LUSC histologies in NSCLC. This gene panel included 91 NSCLC related genes and 4 housekeeping genes. For each sample, original expression of all genes were log2 transformed, normalized against the mean of the 4 housekeeping genes, and z-transformed. Cross-sample normalization was avoided to simplify clinical implementation.

To validate the feasibility of the FNA-nanoString platform, harmonized RNA-Seq data of the TCGA' LUAD (n = 594) and LUSC (n = 551) cohorts were downloaded from National Cancer Institute's Genomic Data Commons (GDC; total sample size: 1145; version: Data Release 8.0; data extraction date: September 7, 2017) [15]. Briefly, the normalized TCGA RNA-Seq data in the format of FPKM-UQ (Fragments Per Kilobase of transcript per Million mapped reads Upper Quartile normalization) of the 91 NSCLC feature genes and the 4 housekeeping genes were extracted, log-transformed, and patient-wisely normalized against housekeeping genes, following the same data normalization approach used in the FNA-nanoString platform. The normalized TCGA data was compared with the FNA-nanoString data and the concordance of the gene expression patterns was analyzed for each NSCLC subtype and for the differential gene expressions between the two subtypes.

Table 1
Patient Characteristics.

Age, mean (range)	65.6 (30–85)
Gender, Females n(%)	59 (42%)
Ethnicity n(%)	124 (89%)
Caucasian	15 (10%)
African American	1 (1%)
Native American	
Tobacco Use n(%)	93 (66%)
Former or Current, mean pack-years (range)	40 (5–168)
Type of Lung Cancer n(%)	55 (47%)
Adenocarcinoma	27 (23%)
Squamous	19 (16%)
NOS	13 (11%)
Small Cell Neuroendocrine	3 (3%)

NOS, Not otherwise specified.

3. Results

Of the 140 patients consented and accrued in the study 39 patients had a research biopsy specimen obtained but were excluded from the final analysis for the following reasons: 13 discovered to have small cell cancer, 3 with neuroendocrine carcinoma, 10 had non-diagnostic needle biopsies, 5 had biopsy confirmed non-cancerous etiologies, 7 were not a primary thoracic malignancy, and 1 case was excluded for unknown reasons (Fig. 1). The remaining 101 samples were analyzed in the study.

3.1. Demographics

Table 1 displays the demographics and smoking status. Age, smoking status, and pack years consumed among patients undergoing the various small volume biopsies were not different. The average age of our patient's undergoing biopsy was 65.6 years and the majority 77.2% were smokers and had smoked approximately 40 pack years.

3.2. Biopsy modality

Evaluation of the type of biopsy modality performed prior to exclusions revealed: EBUS 52 patients (37%), TTNB 55 patients (39%), cTBNA 31 patients (22%), ultrasound guided needle biopsy 1 patient (1%) and EUS 1 patient (1%). TTNB procedures accounted for the majority of non-lung malignancies identified with 5 (13% of total exclusions), and EBUS accounted for the majority of small cell exclusions with 7 (18% of total exclusions). Non-diagnostic results and identification of non-malignant etiologies were similar among biopsy modalities.

3.3. Cytopathology

After exclusions, histology classification of the 101 patients revealed adenocarcinoma (n = 52, 51%), squamous cell (n = 27, 27%), NSCLC not otherwise specified (n = 22, 22%). Comparison of the research pathologic read to the clinical pathologic read showed 86% complete consistency. Of note, there was complete uniformity in the establishment of NSCLC across all reads. The 14 cases (14%) of discordant cases reflected differences in subtyping of NSCLC, a well-known diagnostic challenge in small cytological biopsies (Table 2 supplement).

Evaluation of the type of biopsy performed, compared to the histologic subtype, revealed that adenocarcinoma was most commonly acquired from EBUS and TTFNA biopsies, with cTBNA having equal representation between adenocarcinoma and squamous cell. Small cell carcinoma was also more commonly identified with EBUS and cTBNA as opposed to TTFNA at the time of diagnosis (Fig. 2).

3.4. RNA extraction

RNA extraction was successfully attempted on all 101 samples, but not all samples reached the required threshold to submit for genetic profiling. The total RNA mass collected per sample (Supplement Fig. S3) and %-mass over 300bp per sample (Supplement Fig. S4) demonstrate the high number of samples meeting the predetermined acceptability criteria established by NanoString technologies to attempt gene expression evaluation [13]. Ninety-seven samples (96%) had adequate RNA from one single needle pass to complete genetic profiling with the NanoString® platform. The four aspirates that did not qualify all had insufficient total RNA based on a requirement of 100 ηg of total RNA.

RNA Integrity numbers (RIN) as a grade of the quality of RNA collected were assigned using the Agilent 2100 bioanalyzer with a range of 1–10. RIN values near 10 represent perfectly collected RNA, and values above 8 reflect high quality (intact) RNA suitable for most all RNA applications. Evaluation of the collected RNA data from our 101 RNA samples revealed that the mean RIN for all samples submitted was 4.5 + 1.8, total mass revealed a mean of 1645ng + 2298 with a median of 942 ng, and a mean %mass > 300bp was 84.8% + 8.5 with a median of 86%. Further breakdown of RIN values by biopsy modality revealed no difference in RIN or RNA mass by biopsy modality, (Table 2).

Pooling bronchoscopy biopsy data compared to trans-thoracic biopsy data revealed mean bronchoscopy RIN values of 4.3 + 1.9 and TTFNA RIN values of 4.9 + 1.6 (p = 0.09). Evaluating mean total RNA mass collected by bronchoscopy biopsies was 1738 + 2581ηg compared to TTFNA biopsies 1537 + 1858ηg (p = 0.68). There was no difference in %mass over 300BP with bronchoscopy biopsies 84.2 + 9.1 vs. TTFNA biopsies of 85.8 + 7.6 (p = 0.36).

Evaluating RNA results by histology type revealed the following results: Adenocarcinoma average RIN 4.6 + 1.9, total mass 1532 + 1998ηg and %mass > 300bp was 85%. Squamous cell revealed a RIN of 4.3 + 1.9, total mass 1345 + 1940ηg and %mass > 300bp was 83%. NSCLC NOS revealed a RIN of 4.5 + 1.6, total mass 2397 + 3338ηg and %mass > 300bp was 86%. Evaluation of the RNA characteristics per cancer type showed no difference in RIN, total mass, or %mass based on cellular cancer type.

3.5. Gene expression and histology recapitulation

The adenocarcinoma and squamous cell NSCLC gene panel was generated from microarray data and were validated by applying the genes that had the most significant and strongest difference in expression between adenocarcinoma and squamous cell carcinoma. The normalized expression data using the QuantiGene® platform showed concordance to the tumor histology phenotype from the clinical surgical samples. We then generated transcriptome data using the RNA from the small volume FNAs. We utilized the NanoString platform and were able to recapitulate histology on the majority of the samples with pathologic concordance.

To validate whether the FNA-derived RNAs as well as the nanoString-generated transcriptomics data could recapture the transcriptomic patterns of NSCLC, we performed concordance analysis using the TCGA's RNA-Seq data from the LUAD (lung cancer, adenocarcinoma) and LUSC (lung cancer, squamous cell carcinoma) cohorts. The gene expression patterns in the LUAD (Supplement Fig. S1) and LUSC (Supplement Fig. S2) were highly consistent between our and TCGA transcriptomics data. The intraclass correlation (ICC) coefficients were 0.801 (95% CI: 0.7131147, 0.8646188; p-value: 8e-21) and 0.776 (95% CI: 0.6787765, 0.8467928; p-value: 1.4e-18) for the LUAD and LUSC subtypes, respectively. Such ICC coefficients fell in the range of "excellent concordance" [14]. The Pearson's correlation coefficients were 0.801 (95% CI: 0.7131147, 0.8646188, p-value: < 2.2e-16) and 0.776 (95% CI: 0.6787765, 0.8467928; p-value: < 2.2e-16) for LUAD and LUSC subtypes, respectively.

Table 2
RNA quality by biopsy method.

	All Modalities	cTBNA	EBUS-TBNA	TTNB	p value
RIN	4.5 + 1.8	4.4 + 2.0	4.3 + 1.9	4.9 + 1.6	0.26
Total Mass, mean + SD ng	1645 + 2298	990 + 1072	2223 + 3122	1536 + 1857	0.35
% mass > 300 bp, mean + SD	84.8 + 8.5	84.8 + 8.5	83.0 + 9.2	84.8 + 8.5	0.44

cTBNA, conventional transbronchial needle aspiration, EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration, TTNB, transthoracic needle biopsy, SD, standard deviation.

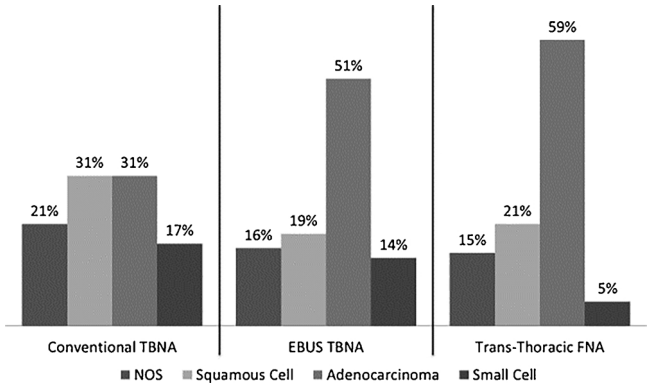

Fig. 2. Malignant cell types identified by biopsy modality. Adenocarcinoma was most commonly acquired from EBUS and TTFNA biopsies, with cTBNA having equal representation between adenocarcinoma and squamous cell.

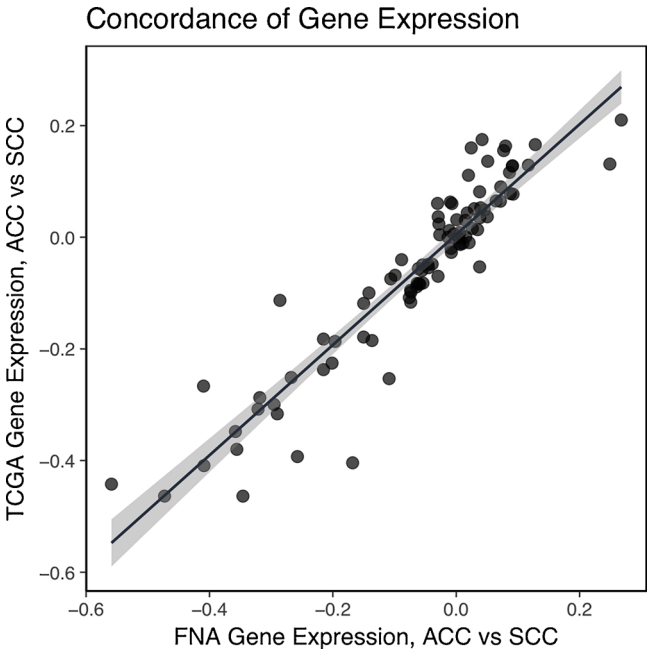

Fig. 3. Concordance between the transcriptomic landscapes of the differential gene expressions, LUAD vs LUSC. Each circle represented one of the 91 genes from the NSCLC gene panel. The x- and y- axes represented the differential expression of the gene according to the transcriptomic data generated from the WFBH NSCLC cohort using FNA technology on nanoString platform and from the TCGA's LUAD and LUSC cohorts generated from RNA-Seq data. The trend line was determined by the linear regression, with the 95% confidence interval labeled with the gray shade.

We further examined whether the FNA-derived transcriptomics could reveal similar differential gene expression patterns as traditional, bulk-tumor-based data. We compared the differential gene expressions of LUAD vs. LUSC between our and TCGA data, using the 91 genes in the NSCLC gene panel as examples. High concordance was observed (Fig. 3), with ICC of 0.927 (CI: 0.892 – 0.951; p-value: 7.15e-41) and Pearson correlation coefficient of 0.9291096 (CI: 0.8942814 – 0.9527502; p-value: 2.2e-16).

Finally, we interrogated the system-specific patterns between the FNA-nanoString and the bulk-RNA-Seq platforms. Lineal regressions of the LUAD (Supplement Table S1) and the LUSC (Supplement Table S2) cohorts, respectively, suggested consistent linear difference between the two platforms. The linear regression intercepts (0.42645 and 0.45871) and coefficients (0.66716 and 0.63610) for the LUAD and LUSC subtypes, respectively, were similar. The linear regression of the differential gene expressions (LUAD vs. LUSC) showed no system-specific difference, with intercept of 0.004776 and coefficient of 0.988255.

4. Discussion

Our data set is the first that prospectively demonstrates that a single dedicated needle aspirate can provide RNA of adequate quantity and quality for gene expression analysis. Although our mean RIN values are lower than typically desired for RNA assessment, newer methodologies have advanced the ability to work with more degraded RNA with lower RIN values. Fresh frozen paraffin embedded (FFPE) samples most accurately make this point as historically they have proven difficult to obtain high quality RNA from, but the use of novel gene probes such as the NanoString® platform are designed to work amongst these difficulties. As a proof of principle, we were able to show that genes taken from microarray data, then validated on surgical samples in a QuantiGene® platform, could then be applied to RNA obtained from small volume lung biopsies that can recapitulate NSCLC histology using a third assay. This confirms the ability of platforms like Nanostring to use small volume samples from lung cancer to perform well for the purpose of biomarker development.

High consistency was observed between the FNA-nanoString and the TCGA's bulk-RNA-Seq platforms in profiling the transcriptomic landscapes of NSCLC. The subtype-specific concordance analysis suggested that the FNA-nanoString platform accurately re-captured the gene expression patterns observed in public data using bulk tumor tissues and RNA-Seq technology. Subtype-specific comparison between the FNA-nanoString and the bulk-RNA-Seq platforms suggested that the systematic difference was linear and independent to tumor subtypes. This was further confirmed by the practically identical differential gene expression patterns observed from the two platforms. The high consistency between the FNA-nanoString and the traditional bulk-RNA-Seq platforms, the linearity of the gene expression shifts from the traditional platform, and the independency to tumor subtypes are crucial for clinical and research applications of our FNA-nanoString platform. Discovered molecular markers and signatures from traditional platforms can be quantitatively translated to our FNA-nanoString platform and directly implemented in clinics for precision diagnosis, therapeutics, and prognosis. Thus, the FNA-nanoString platform bridges the gap between the biomedical research at bench-side and the precision medicine at the bed-side and paves the way to translating basic science to clinical practices.

Our data set demonstrates no meaningful difference in EBUS, conventional, or transthoracic biopsies in the quantity or quality of RNA obtained. The type of tumor (adenocarcinoma vs squamous vs NOS)

also does not affect RNA values and there was no significant difference in the percentage of NOS specimens based on biopsy modality.

Pathology review of NSCLC cytology discrepancies seen between the research and clinical interpretations in NSCLC-NOS underscore the difficulties physicians can face in establishing a pathological diagnosis. Previous studies have demonstrated the challenge of subtyping NSCLC in small volume cytological specimens and underscores the need for an objective test to determine cell type consistently. While agreement is commonly high for the separation of small cell and NSCLC, decreased concordance is observed with further subtyping of NSCLC. Decreased concordance has been associated with more poorly differentiated tumors, limited cytological material, and the absence of definitive immunohistochemical stains [16,17]. All of these factors were involved in the discordant cases in our own study, but despite this complete agreement was common in our series (86%), and in no cases did the observed disagreements alter treatment decisions.

Prior studies completed to evaluate RNA acquisition for translational purposes from a single small volume aspirate have been small and retrospective. Our study is novel in the RNA arena in that previous studies used archived material rather than a prospectively obtained dedicated needle aspirate [9–11]. Schmid-Bindert et al reported on 106 prospective samples; however, they split the entire conglomerate of aspirates for their study as opposed to a single pass [12]. Thus, we are the largest sample size to date using a prospective single needle aspirate from different small volume biopsy modalities to evaluate RNA quality and quantity.

A limitation of our study was the inability to classify the integrity of our collected RNA with 260/280 absorbance ratios to help identify protein contamination or a 260/230 ratio that can help identify contamination with organic compounds, such as phenol, and guanidinium salts. The inability to provide this information was due to the limitations of the spectrophotometer used during the time of RNA processing. However, we employed a high sensitivity bioanalyzer trace of our collected RNA to determine mass and this was acknowledged and acceptable to NanoString Technologies. Additional study limitations include our single-center site and limiting RNA acquisition to a single needle pass.

Despite these study limitations our findings have implications to the study of lung cancer and the limitations presented by minimally invasive small volume biopsies. Current clinical practice presents many competing demands for tumor tissue, as immunohistochemical markers and molecular characterization of EGFR mutations, ALK and ROS rearrangements, with PDL-1 testing are all being requested. We demonstrate that RNA isolated from a small volume biopsy in lung cancer patients was variable in quantity but had adequate overall quality for the evaluation of gene expression, regardless of the type of minimally invasive biopsy modality employed.

In this study, RNA retrieval from a single-pass FNA regardless of procedural approach showed equivalence and suitability for gene expression assessments. RNA extraction has the potential to help small volume samples maximize their ability to provide valuable material for genetic profiling. One important downstream effect of this demand on tissue sample is the realization that limited cytological material is being consumed for an ever-growing list of biomarkers with the stark realization that prioritization is now required to provide the most important results for patients who have sparse samples. Further studies are needed to test RNA for subtyping of NSCLC and detecting specific actionable genetic mutations.

Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.lungcan.2018.11.023.

References

[1] M.K. Gould, J. Donington, W.R. Lynch, P.J. Mazzone, D.E. Midthun, D.P. Naidich, R.S. Wiener, Evaluation of individuals with pulmonary nodules: when is it lung cancer? Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines, Chest 143 (Suppl. 5) (2013) e93S–e120S.

[2] N. Rekhtman, Commentary on testing of non-adenocarcinomas, Arch. Pathol. Lab. Med. 142 (7) (2018) 798.

[3] S. Murgu, H. Colt, Role of the pulmonologist in ordering post-procedure molecular markers in non-small-cell lung cancer: implications for personalized medicine, Clin. Lung Cancer 14 (6) (2013) 609–626.

[4] W.J. Gradishar, B.O. Anderson, R. Balassanian, S.L. Blair, H.J. Burstein, A. Cyr, A.D. Elias, W.B. Farrar, A. Forero, S.H. Giordano, M.P. Goetz, L.J. Goldstein, S.J. Isakoff, J. Lyons, P.K. Marcom, I.A. Mayer, B. McCormick, M.S. Moran, R.M. O'Regan, S.A. Patel, L.J. Pierce, E.C. Reed, K.E. Salerno, L.S. Schwartzberg, A. Sitapati, K.L. Smith, M.L. Smith, H. Soliman, G. Somlo, M.L. Telli, J.H. Ward, R. Kumar, D.A. Shead, Breast cancer, version 4.2017, NCCN clinical practice guidelines in oncology, J. Compr. Cancer Netw. 16 (3) (2018) 310–320.

[5] L.N. Harris, N. Ismaila, L.M. McShane, D.F. Hayes, Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline Summary, J. Oncol. Pract. 12 (4) (2016) 384–389.

[6] G.A. Silvestri, A. Vachani, D. Whitney, M. Elashoff, K. Porta Smith, J.S. Ferguson, E. Parsons, N. Mitra, J. Brody, M.E. Lenburg, A. Spira, A.S. Team, A bronchial genomic classifier for the diagnostic evaluation of lung Cancer, N. Engl. J. Med. 373 (3) (2015) 243–251.

[7] T. Nakajima, T. Anayama, T. Waddell, S. Keshavjee, I. Yoshino, K. Yasufuku, Extraction of RNA using fine-needle aspiration samples stored under different conditions: a pilot study, J. Bronchology Interv. Pulmonol. 18 (3) (2011) 218–222.

[8] R. Lee, D.J. Cousins, E. Ortiz-Zapater, R. Breen, E. McLean, G. Santis, Gene expression profiling of endobronchial ultrasound (EBUS)-derived cytological fine needle aspirates from hilar and mediastinal lymph nodes in non-small cell lung cancer, Cytopathology 24 (6) (2013) 351–355.

[9] T. Nakajima, T. Anayama, T. Koike, T. Waddell, S. Keshavjee, H. Kimura, I. Yoshino, K. Yasufuku, Simultaneous isolation of total RNA, DNA, and protein using samples obtained by EBUS-TBNA, J. Bronchology Interv. Pulmonol. 18 (4) (2011) 301–305.

[10] T. Nakajima, R. Zamel, T. Anayama, H. Kimura, I. Yoshino, S. Keshavjee, K. Yasufuku, Ribonucleic acid microarray analysis from lymph node samples obtained by endobronchial ultrasonography-guided transbronchial needle aspiration, Ann. Thorac. Surg. 94 (6) (2012) 2097–2101.

[11] D. Petriella, D. Galetta, V. Rubini, E. Savino, A. Paradiso, G. Simone, S. Tommasi, Molecular profiling of thin-prep FNA samples in assisting clinical management of non-small-cell lung cancer, Mol. Biotechnol. 54 (3) (2013) 913–919.

[12] G. Schmid-Bindert, Y. Wang, H. Jiang, H. Sun, T. Henzler, H. Wang, L.R. Pilz, S. Ren, C. Zhou, EBUS-TBNA provides highest RNA yield for multiple biomarker testing from routinely obtained small biopsies in non-small cell lung cancer patients - a comparative study of three different minimal invasive sampling methods, PLoS One 8 (10) (2013) e77948.

[13] Proof of Principle Sample Submission Guidelines, NanoString Technologies, 2017, p. 3.

[14] R. Kwiecien, A. Kopp-Schneider, M. Blettner, Concordance analysis: part 16 of a series on evaluation of scientific publications, Arztebl. Int. 108 (30) (2011) 515–521.

[15] M.A. Jensen, V. Ferretti, R.L. Grossman, L.M. Staudt, The NCI Genomic Data Commons as an engine for precision medicine, Blood 130 (4) (2017) 453–459.

[16] M.F. Zakowski, N. Rekhtman, M. Auger, C.N. Booth, B. Crothers, M. Ghofrani, W. Khalbuss, R. Laucirica, A.T. Moriarty, Z.L. Tabatabai, G.A. Barkan, Morphologic accuracy in differentiating primary lung adenocarcinoma from squamous cell carcinoma in cytology specimens, Arch. Pathol. Lab. Med. 140 (10) (2016) 1116–1120.

[17] D.P. Steinfort, P.A. Russell, A. Tsui, G. White, G. Wright, L.B. Irving, Interobserver agreement in determining non-small cell lung cancer subtype in specimens acquired by EBUS-TBNA, Eur. Respir. J. 40 (3) (2012) 699–705.