Case Report: Metabolic Profiling Identifies Lung Tumor Responsiveness to Erlotinib

Teresa W-M Fan[a,b,c], **Andrew N Lane**[b,c], **Richard M Higashi**[a,c], **Michael Bousamra II**[d], **Goetz Kloecker**[c], and **Donald M. Miller**[b,c]

[a] Department of Chemistry University of Louisville, 2205 S. Brooks St., Louisville KY40208

[b] Center for Regulatory and Environmental Analytical Metabolomics (CREAM)

[c] J.G. Brown Cancer Center, Department of Medicine, University of Louisville, 529 S. Jackson St., Louisville KY 40202

[d] Department of Surgery, University of Louisville, KY 40202

Abstract

A subtype of non-small cell lung cancer, bronchioalveolar adenocarcinoma (BAC), is more prevalent in Asian female non-smokers, and is more likely to respond to treatment with tyrosine kinase inhibitors such as erlotinib and gefitinib. Nuclear magnetic resonance and mass spectrometry-based metabolomic analysis of extracts from two different lung lesions and surrounding non-cancerous tissues of a BAC patient showed novel protein and phospholipid-associated metabolic differences that correlated with tumor development as well as PET and erlotinib sensitivity.

Keywords

bronchioalveolar adenocarcinoma; metabolomics; erlotinib

Introduction

Non-small cell lung cancer (NSCLC) accounts for roughly 85% of all lung cancers, most of which are tobacco-related. However, 10–15 % of lung cancer occur in nonsmokers and disproportionately in women (Wakelee et al. 2007). Among these is the slow-growing bronchioalveolar adenocarcinoma (BAC) subtype of NSCLC. Regardless of the etiology or subtypes, early stage lung cancer is generally difficult to detect and the median 5-year survival rate (ca. 15%) has barely changed in the last 20 years (Pirozynski 2006).

Current chemotherapies have not been as successful for NSCLC as for other solid tumors (Collaboration 2009). Recently, targeted therapies that inhibit tyrosine kinase receptors (including EGFR) have been developed. These agents block the signaling pathways that are important in stimulating tumor cell proliferation. Two agents in particular have gone through phase II/III clinical trials, namely gefitinib and erlotinib. Although the overall response rate in

NSCLC to these agents has been disappointing, there is a sub-population that responds well to these inhibitors, namely those that carry a gain of function mutation in the EGFR. These mutations are found more frequently in females of Asian origin with the bronchioalveolar subtype of NSCLC (Ahmed et al. 2006; Giaccone et al. 2006; Kris et al. 2006; Raz et al. 2006; Riely et al. 2006).

Early and sensitive detection of lung cancer coupled with appropriate treatment may significantly improve overall survival from the disease. This may be explored through distinct metabolic activities of lung cancer cells, which are likely to precede any morphological change. The metabolism of cancer cells is altered to meet the demand for accelerated and uncontrolled growth. For example, many tumors have greatly enhanced glucose uptake and glycolysis as observed by PET detection, even under aerobic conditions (i.e. the Warburg effect (Warburg 1956;Lu et al. 2002; Altenberg et al. 2004; Garber 2004), leading to increased lactate secretion. Further, increased anabolic activity is required for the biosynthesis of macromolecules (protein, carbohydrate, lipids and nucleic acids) essential to cancer cell growth.

Although there has been extensive genetic analysis of lung tumors (Bhattacharjee et al. 2001; Brognard et al. 2001; Giaccone et al. 2006; Carretero et al. 2007; Il Na et al. 2007; Sakuma et al. 2007), the *in situ* metabolic differences between cancerous and normal lung tissues have not been systematically characterized. We have addressed this problem by developing NMR and mass spectrometry (MS)-based technologies to determine the profile of a wide range of metabolites and their biosynthetic pathways in lung cancer cells and tissues (Fan et al. 2005; Fan et al. 2006) and in human subjects (Lane et al. 2009) (and T. WM. Fan, A. N Lane, et al. submitted). Here we report the use of these technologies to compare the metabolic profiles of a stage I bronchioalveolar, erlotinib-sensitive carcinoma, and an erlotinib-insensitive, slower-growing (possibly earlier developmental) lesion against normal lung tissue from the same patient. Many previously unknown, drug-dependent and -independent metabolic distinctions were thereby discovered.

Results

An 80-year old Asian female, never smoker was found to have two lung lesions. The PET scan (Figure 1) showed that the mass in the lower lobe (3.5×3.0 ×1.8 cm) was very active in glucose accumulation and a small nodule in the upper lobe (1.8×1.2×1.3 cm) was barely observable above background. Needle biopsy of the lower lobe lesion revealed bronchioalveolar adenocarcinoma (BAC) of pulmonary origin. After treatment with a daily dose of 150 mg of Erlotinib for one month, the lower lobe lesion was reduced by >50% in size, whereas the upper lobe lesion showed no response to the drug.

Lobectomy of the lower lobe (LL) and a wedge resection of the upper lobe (UL) lesion were performed and well tolerated by the patient. Pleural effusion and lymph nodes were clear of detectable cancer cells. A slice of each tumor was immediately flash frozen in liquid nitrogen (< 2 minutes after excision). A second slice was preserved in formalin for histopathology. A slice of normal tissue taken from the lobe > 4 cm from the cancerous margins was similarly treated. Blood samples were also collected pre- and post-operatively, immediately placed on ice, and centrifuged at 4 °C to separate the red cells from the plasma. Plasma was flash frozen in liquid nitrogen for storage at −80 °C with a total processing time of < 30 minutes. These protocols were developed to minimize artifactual metabolic changes during processing (Fan et al., submitted).

Pathology

Pathological examination of H&E stained tissue slices indicated both lesions to be well-differentiated adenocarcinomas with BAC-like features. The PET-positive, erlotinib-

responsive LL lesion is a grade I BAC, whereas the weakly PET-positive nodule of UL was also a BAC-like tumor which may be independent of the LL lesion. Sections were also stained for EGFR using anti EGFR mAb, which showed low EGFR expression for the normal tissue, high expression in the UL lesion, and near normal expression in the LL lesion (possibly the result of Erlotinib treatment).

Metabolomics analysis

Polar metabolites from tissues and plasma were extracted according to our established protocols (Fan et al. 2005; Fan et al. 2008; Fan et al. 2008; Lane et al. 2008). Metabolites were identified and quantified using high resolution one and two-dimensional NMR spectroscopy as well as by GC-MS, as described previously (Fan et al. 1986; Fan et al. 2008; Lane et al. 2008). Figure 2 compares the one-dimensional ^{1}H NMR spectra of normal versus lower lobe (LL) and upper lobe (UL) nodules. The three tissues showed significantly different ^{1}H NMR profiles, particularly in terms of lactate, amino acids, phosphocholine, (P-choline), choline, and glucose. Metabolites profile had the general order of LL tumor ≥ UL lesion > normal tissue except for glucose and glycogen with a reverse order. Many of these metabolites were quantified by GC-MS and ^{1}H NMR, as shown in Figure 3. The GC-MS analysis verified the semi-quantitative trend observed by ^{1}H NMR in Figure 2. It is also clear that many of the metabolites (e.g. P-choline, Ser, OHPro, Asn, α-glycerol-3-phosphate or α-G3P, citrate, fumarate, malate, succinate) in the UL lesion were closer in concentration to the LL tumor than the normal lung tissue. However, lactate and glucose levels in the UL lesion exhibited more of the normal tissue type (Figure 3).

Discussion

Increased accumulation of lactate in the LL lesion is typical of malignant tumors and suggests the presence of accelerated glycolysis or Warburg effect. The depletion of glucose and glycogen in this lesion is presumably a result of this enhanced glycolysis, which is again linked to the much higher lactate content or accumulation of ^{18}F-deoxyglucose-6-phosphate, as reflected by its brighter PET image density (Figure 1). By similar reasoning, the weak PET image density of the UL lesion is consistent with its near normal glucose and lactate profile. Thus, the UL lesion may not have undergone transformations into an enhanced glycolytic state, which differentiates its development from the LL lesion.

In contrast, some amino acids (e.g. Glu, Asp, Ser, OHPro, Met, Asn), P-choline, α-G3P, citrate, malate, fumarate, and succinate in the UL lesion accumulated to levels closer to the LL lesion than normal tissues. Cancer-associated accumulation of these metabolites was also observed in over 30 other non-BAC NSCLC cancer subjects (Fan et al., submitted), which suggests that metabolic transformations governing the accumulation have occurred in both UL and LL lesions. These transformations are likely to be involved in the uptake of amino acids and choline, choline phosphorylation, α-G3P production, as well as Krebs cycle function, all of which are essential to the synthesis of proteins and phospholipids. These processes appear to have preceded enhanced glucose uptake and glycolysis in the process of tumorigenesis, which is common to the LL lesion and later stage tumors from 20 other NSCLC patients. Thus, this case study suggests that deregulation of protein and phospholipid-associated metabolism could be early transforming events in BAC development, and demonstrates that metabolomic analysis may be valuable for differentiating early stage erlotinib-sensitive and insensitive NSCLC.

Acknowledgments

Abbreviations used

BAC

Bronchioalveolar Adenocarcinoma

NSCLC

Non Small Cell lung Cancer

EGFR

Epidermal Growth Factor Receptor

MS

Mass Spectrometry

PET

positron emission tomography

References

Ahmed SM, Salgia R. Epidermal growth factor receptor mutations and susceptibility to targeted therapy in lung cancer. Respirology 2006;11:687–692. [PubMed: 17052295]

Altenberg B, Greulich KO. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. Genomics 2004;84:1014–1020. [PubMed: 15533718]

Bhattacharjee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, Ladd C, Beheshti J, Bueno R, Gillette M, Loda M, Weber G, Mark EJ, Lander ES, Wong W, Johnson BE, Golub TR, Sugarbaker DJ, Meyerson M. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Proceedings of the National Academy of Sciences of the United States of America 2001;98:13790–13795. [PubMed: 11707567]

Brognard J, Clark AS, Ni YC, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Research 2001;61:3986–3997. [PubMed: 11358816]

Carretero J, Medina PP, Blanco R, Smit L, Tang M, Roncador G, Maestre L, Conde E, Lopez-Rios F, Clevers HC, Sanchez-Cespedes M. Dysfunctional AMPK activity, signalling through mTOR and survival in response to energetic stress in LKB1-deficient lung cancer. Oncogene 2007;26:1616–1625. [PubMed: 16953221]

Collaboration, TC. Library. John Wiley and Sons, Ltd; 2009. Chemotherapy for non-small cell lung cancer C; p. 1-106.

Fan T, Bandura L, Higashi R, Lane A. Metabolomics-edited transcriptomics analysis of Se anticancer action in human lung cancer cells. Metabolomics 2005;1:1–15.

Fan TW, Lane AN. Structure-based profiling of Metabolites and Isotopomers by NMR. Progress in NMR Spectroscopy 2008;52:69–117.

Fan TWM, Higashi RM, Lane AN. Integrating metabolomics and transcriptomics for probing Se anticancer mechanisms. Drug Metabolism Reviews 2006;38:707–732. [PubMed: 17145697]

Fan TWM, Higashi RM, Lane AN, Jardetzky O. Combined Use of H-1-NMR and GC-MS for Metabolite Monitoring and Invivo H-1-Nmr Assignments. Biochimica Et Biophysica Acta 1986;882:154–167. [PubMed: 3011112]

Garber K. Energy boost: The Warburg effect returns in a new theory of cancer. Journal of the National Cancer Institute 2004;96:1805–1806. [PubMed: 15601632]

Giaccone G, Ruiz MG, Le Chevalier T, Thatcher N, Smit E, Rodriguez JA, Janne P, Oulid-Aissa D, Soria JC. Erlotinib for frontline treatment of advanced non-small cell lung cancer: a phase II study. Clinical Cancer Research 2006;12:6049–6055. [PubMed: 17062680]

Na, IIl; Rho, JK.; Choi, YJ.; Kim, CH.; Park, JH.; Koh, JS.; Ryoo, BY.; Yang, SH.; Lee, JC. The survival outcomes of patients with resected non-small cell lung cancer differ according to EGFR mutations and the P21 expression. Lung Cancer 2007;57:96–102. [PubMed: 17337084]

Kris MG, Giaccone G, Davies A, Fukuoka M, Garfield DH, Jassem J, Quoix EA, Sandler AB, Scagliotti GV, Van Meerbeeck JP, West H. Systemic therapy of bronchioloalveolar carcinoma: Results of the first IASLC/ASCO consensus conference on bronchioloalveolar carcinoma. Journal of Thoracic Oncology 2006;1:S32–S36. [PubMed: 17409999]

Lane, AN.; Fan, TW.; Higashi, RM. Isotopomer-based metabolomic analysis by NMR and mass spectrometry. In: John, HWD.; Correia, J., editors. Biophysical Tools for Biologists, Vol 1. Vol. 84. San Diego: Academic Press; 2008. p. 541-588.

Lane AN, Fan TW, Higashi RM, Tan J, Bousamra M, Miller DM. Prospects for clinical cancer metabolomics using stable isotope tracers. J Exp Molec Pathol. 2009In Press

Lu HS, Forbes RA, Verma A. Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. Journal of Biological Chemistry 2002;277:23111–23115. [PubMed: 11943784]

Pirozynski M. 100 years of lung cancer. Respiratory Medicine 2006;100:2073–2084. [PubMed: 17056245]

Raz DJ, He BA, Rosell R, Jablons DM. Bronchioloalveolar carcinoma: A review. Clinical Lung Cancer 2006;7:313–322. [PubMed: 16640802]

Riely GJ, Pao W, Pham DK, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M, Miller VA. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clinical Cancer Research 2006;12:839–844. [PubMed: 16467097]

Sakuma Y, Matsukuma S, Yoshihara M, Nakamura Y, Noda K, Nakayama H, Kameda Y, Tsuchiya E, Miyagi Y. Distinctive evaluation of nonmucinous and mucinous subtypes of bronchioloalveolar carcinomas in EGFR and K-ras gene-mutation analyses for Japanese lung adenocarcinomas - Confirmation of the correlations with histologic subtypes and gene mutations. American Journal of Clinical Pathology 2007;128:100–108. [PubMed: 17580276]

Wakelee HA, Chang ET, Gomez SL, Keegan TH, Feskanich D, Clarke CA, Holmberg L, Yong LC, Kolonel LN, Gould MK, West DW. Lung cancer incidence in never smokers. Journal of Clinical Oncology 2007;25:472–478. [PubMed: 17290054]

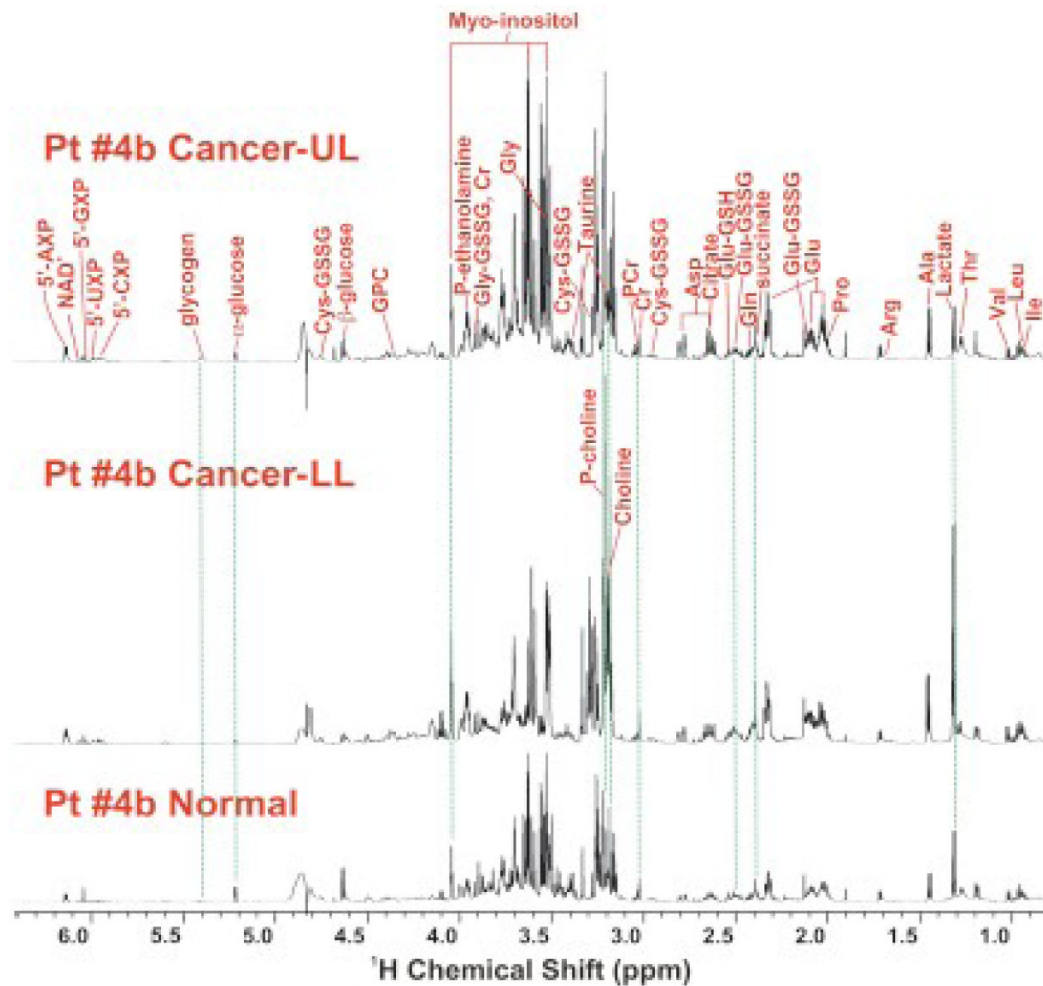Warburg O. On the origin of cancer cells. Science 1956;123:309–314. [PubMed: 13298683]
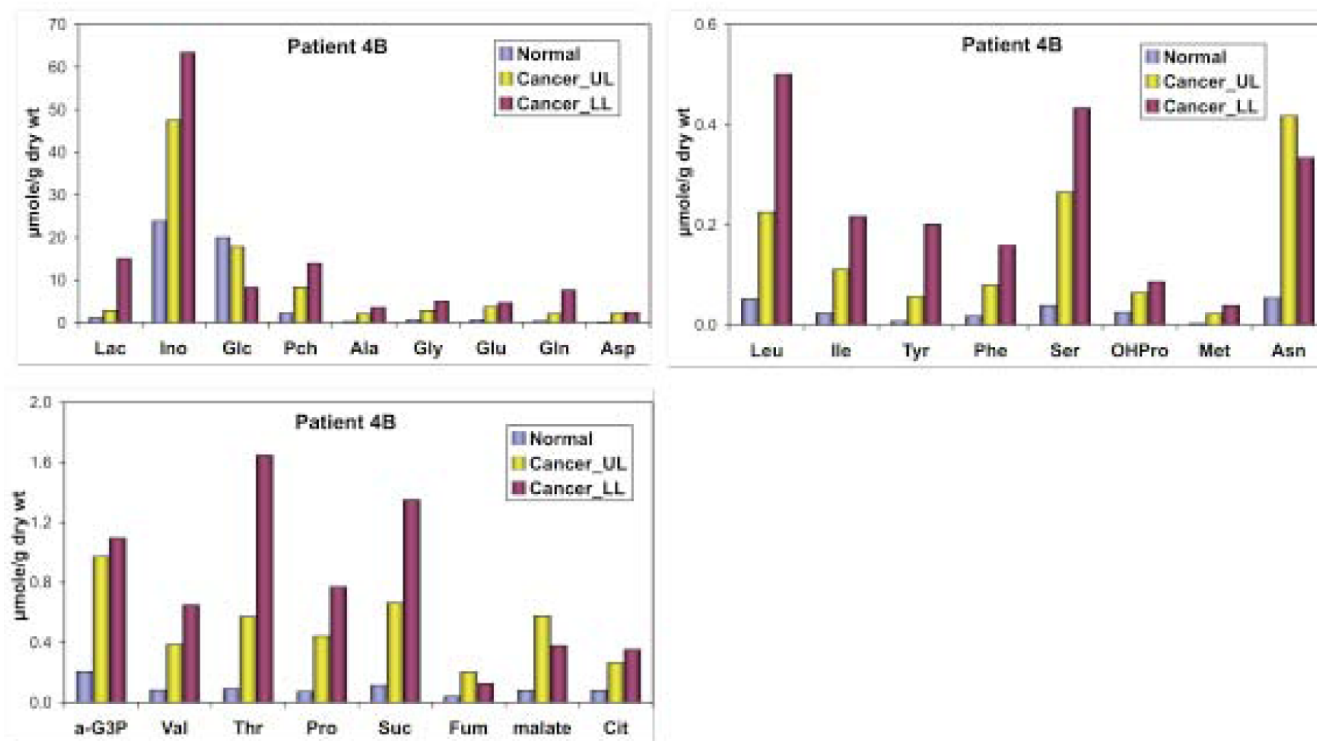
Upper Lobe Nodule
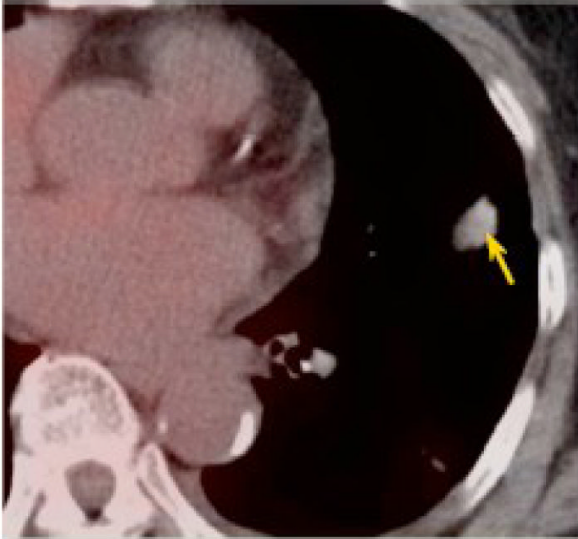
Lower Lobe Nodule
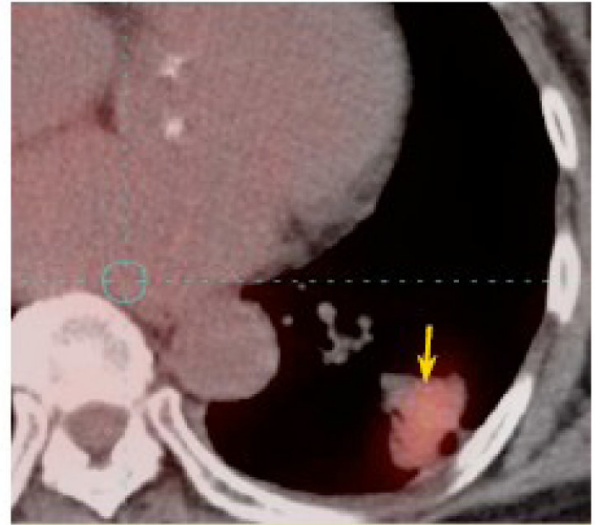
Figure 1.
PET images of upper and lower lobe lung lesions (indicated by arrows) of an 80-year old Asian female patient. Red to white colors indicates high to low density.

Figure 2.
^{1}H NMR spectra of extracts of paired normal and lung lesions.
Flash frozen slices from upper lobe (UL, top panel), lower lobe (LL, middle panel) nodules, and non-cancerous tissues (bottom panel) were taken from the resected lung of the patient shown in Figure 1, and extracted with 10% TCA and ^{1}H NMR spectra of extracts were recorded at 600 MHz. Metabolite assignments were based on our in-house database, 2-D ^{1}H TOCSY and ^{1}H-^{13}C HSQC NMR spectra (not shown) (Fan et al. 2008). The three spectra were normalized in terms of tissue weight and NMR spectral parameters for direct comparison.

Figure 3.
Metabolite concentration in normal, upper and lower lobe lung nodules.
Myo-inositol (Ino), glucose (Glc), phosphocholine (PCh) were quantified from ¹H NMR
spectra in Fig. 1 while the other metabolites were quantified by GC-MS as described previously
(Fan et al. 1986). Lac: lactate; α-G3P: α-glycerol-3-phosphate; Suc: succinate; Fum: fumarate;
Cit: citrate. UL upper lobe, LL lower lobe