Plasma Proteomics Analysis for Screening Anlotinib Responders in Non-small Cell Lung Cancer

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Research

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Abstract

Background

Anlotinib has been demonstrated to be effective in advanced non-small cell lung cancer (NSCLC) patients. The underlying value of proteomics for anlotinib study remains unclear.

Methods

In this study, plasma samples from 28 anlotinib-treated NSCLC patients (including 14 responders and 14 non-responders) were performed proteomics analysis. LC-MS/MS analysis was performed on those samples with different time points including baseline, best response and progression disease. Bioinformatics analysis was performed to understand the underlying value of those differential proteins.

Results

Proteomics analysis suggested the differential proteins from responders after anlotinib administration potential play a role in the molecular mechanism characterization and biomarker screening. The differential proteins between responders and non-responders at baseline mainly contribute to biomarker screening. Integrative analysis indicated 43 proteins could be used as underlying biomarkers for clinical practice. Lastly, we selected ARHGDIB and demonstrated that it has potential predictive value for anlotinib.

Conclusions

This study not only offered the first insight that the proteomic technology potentially be used for anlotinib molecular mechanism characterization, but also provided a basis for anlotinb biomarker screening via proteomics in the future.

1. Background

Anlotinib has been used for 3rd-line or further treatment in patients with advanced non-small cell lung cancer (NSCLC) therapy in China[1, 2]. Clinical evidences have demonstrated that anlotinib significantly prolongs progression-free survival (PFS) (anlotinib vs placebo: 5.37 months vs 1.40 months) and median overall survival (OS) (anlotinib vs placebo: 9.63 months vs 6.30 months)[1]. Recent studies indicated anlotinib not only brings survival benefit to those NSCLC patients, but also enhances the survival time to other type of cancers including small cell lung cancer (SCLC), renal cell carcinoma (RCC), and so on[3–5]. Anlotinib-induced inhibition of angiogenesis and proliferative signaling contributed to the phenomenon[6, 7]. Molecular mechanism study suggested the targets of anlotinib include CCL2 signaling pathway, receptor tyrosine kinases vascular endothelial growth (VEGFR) signaling pathway, endothelial growth factor receptor (EGFR) signaling pathway, fibroblast growth factor receptor (FGFR) signaling pathway, and so on[8, 9]. The characteristics of this multi-target tyrosine kinase inhibitor (TKI) make a great difficult for clinical biomarker screening.

Our molecular mechanism study suggested anlotinib-induced CCL2 decreasing could be used as a predictive factor for stratification[8]. Furthermore, KLK5 and L1CAM levels have potential values for screening anlotinib responders[10]. Importantly, we thought that next generation sequencing (NGS) for plasma cell free DNA (cfDNA) plays a role in anlotinib responsive stratification, and found that the biomarker tumor mutation index (TMI) plus *IDH1*^{exon4} mutation status can significant stratify the anlotinib responders[11]. In addition, other recent studies introduced predictors including CD31-labeled circulating endothelial cells and baseline characteristics of patients, for the stratification of those patients treated with anlotinb[12, 13]. Due to complex architecture of anlotinib anti-angiogenic signaling pathway[14], as far as possible screen biomarker from omics analysis will contribute to multiple cancer (NSCLC, SCLC, RCC, and so on) treatments with anlotinib. Therefore, in the present study, we performed proteomics analysis on the plasma samples from anlotinib clinical trial, and discussed the potential feasibility for anlotinib responder screening.

2. Methods

2.1. Patients selection In the ALTER0303 study (https: //clinicaltrials.gov/ NCT02388919), totally 440 qualified advanced NSCLC patients enrolled and finished the clinical study. Among the sample library, we selected patients according to the following three criteria: 1. The non-responders with best clinical objective response defined as progression disease; the responders with best clinical objective response defined as partial response. 2. The plasma samples must be collected from the same patient at different time points. 3. All plasma samples should be passed the quality control (Supplementary Table 1). Briefly, the qualified plasma samples from 14 anlotinib non-responders at two time points of baseline and progression disease, and the plasma samples from 14 anlotinib responders at three time points of baseline, best response, and progression disease (Fig. 1). The characteristics were shown in Supplementary Table 1 and Supplementary Table 2. All of other administration and clinical care information have been introduced in previous study[1].

2.2. Plasma collection and processing All of these plasma samples collection performed standard procedures. Briefly, peripheral blood from advanced NSCLC patients was collected using EDTA tubes. All of samples performed centrifugation (1600 × g for 10 min) within 2 hours of blood collection. Then, the upper plasma was transferred to 1 mL cleaned Eppendorf tubes using a pipette, and stored at −80°C. Due to limitation of plasma volume, mixed plasma was prepared for proteomics analysis. Detailedly, 7 plasma samples (patient No. 1–7) from responders at baseline were mix together. Each patient contributed 100 μL plasma, and mixed totally 700 μL plasma for protein extraction. As same as previous procedure, other 7 plasma samples (patient No. 8–14) from responders at baseline mixed together as a duplicate for protein extraction (Supplementary Table 1 and Supplementary Table 3). Therefore, we prepared two mixed samples from responders at baseline for proteomics analysis. This study evaluated three time points (including baseline, best response and progression disease) for responders, and two time points (including baseline and progression disease) for non-responders. All samples were collected from the same batch patients at different time points (Supplementary Table 3). Ethical approval for this

study has obtained from institutional ethics committee prior to commencing. The consent for all resources (including patients' clinical data, blood samples, tumor samples, and so on) was obtained before clinical screening.

2.3. Abundant protein depletion 700 μL plasma sample was used for protein extraction. Abundant protein existed in sample was deleted using thermoscientific high-select top 14 abundant protein depletion resin kit (Thermo Fisher, USA) (Supplementary Fig. 1, Supplementary Table 4). Detailed methods were according to the production protocol. After abundant protein depletion, the quantitation of protein samples was performed using bicinchoninic acid (BCA) method.

2.4. Enzymatic hydrolysis and peptide desalination 300 μg protein for each sample was performed enzymatic hydrolysis. Briefly, add DTT to the concentration of 100 mM, and then incubated in 100℃ water for 5 min, and cool to room temperature. After treatment with IAA, NH4HCO3 buffer, and Trypsin buffer, the processing of enzymatic hydrolysis completed. Add some 0.1% TFA solution, peptide desalination using C18 cartridge, and then quantitation of the peptide.

2.5. TMT labelling 100 μg peptide sample was performed TMT labelling using thermo fisher TMT labelling kit (Thermo Fisher, USA). Detailed methods were according to the production protocol. After labelling, equal quantity of peptide tags mixed together. The dry peptides were performed grade separation using pierce high-pH reverse-phase peptide fractionation kit (Thermo Fisher, USA). Lastly, collect the samples and separate as 15 components. Each dry component re-dissolved using 0.1% FA solution, and prepared for LC-MS analysis.

2.6. LC-MS/MS analysis Above 15 components were performed MS analysis serially. HPLC was used for component separation. After separation, Q-Extractive Plus MS (Thermo Scientific, USA) was used for tandem mass spectrometry analysis. Collection of peptides as follows: collection the 20 fragment map after each full scan; First grade MS resolution (70,000 @m/z 200, AGC targe: 1e6, one grade Maximum IT: 50 ms); two grade MS resolution (35,000 @m/z 200, AGC targe: 1e5, one grade Maximum IT: 50 ms); MS2 Activation Type: HCD; Isolation window: 1.6 Th; Normalized collision energy: 35. Raw data were upload to MaxQuant software (version 1.6.0.16), and performed database retrieval (Supplementary Table 5). The database (Uniprot_Human_162254_20180320) website is https://www.uniprot.org/uniprot/?query = organism:9606. All raw data were deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository with the project ID: IPX017919[15].

2.7. Bioinformatics analysis Protein clustering was performed to the differential proteins between different samples. Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed using a public bioinformatics resource platform (DAVID, https://david.ncifcrf.gov/) by uploading the differential gene lists. Under GO analysis, biological process, molecular function, and cellular component were used for charactering differential proteins.

2.8. ARHGDIBfunction analysis Fold-change alteration of *ARHGDIB* mRNA expression in NCI-H1975 cells after anlotinib administration was validated using our previous RNA-seq data (EMBL database under accession number E-MTAB–5997: http://www.ebi.ac.uk/arrayexpress/). Clinical data and RNA-seq data for 997 NSCLC patients were downloaded from the TCGA data portal (https://cancergenome.nih.gov/). All data were parsed using a custom R function. The FPKM expression values were transformed to log2 counts per million using the voom function from the limma R package. Cutoff value for *ARHGDIB* was defined using “ward method” as our previous reported[11].

2.9. Detection of plasma ARHGDIB levels and anlotinib responsive analysis The ELISA kit for ARHGDIB detection was purchased from Cloud-Clone Corporation (CHN). The experimental procedures were performed according to the manufacturer’s protocols. All of plasma samples from 14 responders and 14 non-responders were detected at the time point of baseline. Based on the ARHGDIB content, method of binary bits was used for responsive stratification prediction.

2.10. Statistical analysis PFS and OS were summarized as median values and two-sided 95% confidence interval and were analysed using the Kaplan-Meier method. The Mantel-Cox test was used to perform the Kaplan-Meier survival analysis in GraphPad Prism 5. Differences were considered significant at $P < 0.05$, 1 $P < 0.01$, and $P < 0.001$.

3. Results

3.1. Compare the differential proteins between baseline and best response in anlotinib responders

Plasma samples from 28 patients were used for proteomics analysis. In this cohort, 14 patients without any anlotinib response (Median PFS: 35.5 days; Median OS: 158.5 days), and 14 patients with good anlotinib response (Median PFS: 189 days; Median OS: 373 days) (Fig. 1, Supplementary Table 2). The biomarkers using plasma proteomics to screen responders and non-responders remain unclear. In order to screen the effective plasma biomarkers, we firstly compared the plasma protein levels those from responders between the two time points including baseline and best response. Total 528 proteins were detected via quantitative proteomics. Of 528 proteins, 28 protein levels were relative higher and 30 protein levels were relative lower at the time point of best response, as compared with those from baseline (Fig. 2a). Heat map analysis of those 58 protein differential levels indicated there are some sample differences between the duplicates, but still found lots of proteins showed remarkably differences (Fig. 2b). Here, we performed biological process analysis and cell component analysis on those 58 differential proteins. Large parts of those proteins played roles in single-multicellular organism process and multi-cellular organismal process after biological process analysis, which also played roles in extracellular region part and extracellular region after cell component analysis (Fig. 2c). Furthermore, biological process analysis suggested the up-regulated proteins enriched in the items of single-multicellular organism process, multi-cellular organismal process, biological regulation, and so on, and the down-regulated proteins enriched in the items of protein metabolic process, organonitrogen compound metabolic process, nitrogen compound metabolic process, and so on (Fig. 2d). These results suggested that anlotinib performed its

anti-tumor effect may associated with the 58 differential proteins, and potentially be used for anlotinib biomarker screening.

3.2. Differential proteomics analysis throughout anlotinib administration in anlotinib responders

To analysis the changes of plasma protein levels after anlotinib administration, we compared the plasma protein levels those from responders at three time points including baseline, best response and progression disease. Total 18 proteins were screened out, and showed significantly alteration (Fig. 3a). Heat map analysis indicated 7 protein levels increased at the time point of best response, then decreased at time point of progression disease. 7 protein levels increased continually and 1 protein levels decreased continually after anlotinib administration. 3 protein levels decreased at the time point of best response, and then increased at time point of progression disease (Fig. 3b). Biological process analysis suggested those 18 proteins enriched in the items of platelet activation, cell activation, blood coagulation, and so on. Cell component analysis suggested those proteins enriched in membrane-bounded vesicle, extracellular space, vesicle, and so on (Fig. 3c). KEGG pathway analysis indicated those proteins enriched in the signaling pathways including shigellosis, complement and coagulation cascades, salmonella infection, and salivary secretion (Fig. 3d). These results suggested that anlotinib-induced plasma protein level alterations may affect the different biological processes, cell components, and signaling pathways which were potentially involved in acquired resistance.

3.3. Analysis of resistance to anlotinib via proteomics characterization in non-responders

Next, we compared the plasma protein levels those from non-responders at two time points including baseline and best response. Totally 41 plasma protein levels changed including 20 protein levels significantly enhanced and 21 protein levels remarkably dropped, after administration of anlotinib (Fig. 4a). Biological process analysis suggested those proteins enriched in the items including cell migration, localization of cell, cell motility, and so on (Fig. 4b). Further analysis indicated that the up-regulated proteins enriched in the items of metabolic process, organic substance metabolic process, cellular process, and so on. The down-regulated proteins enriched in the items of endocytosis, receptor-mediated endocytosis, chemical homeostasis, and so on (Fig. 4c). Cell component analysis suggested those proteins enriched in the items including extracellular region, extracellular region part, vesicle, and so on (Fig. 4b). These results provided hypothesis that those proteins may play an important role in compensatory effect of anlotinib-induced tumor cell process inhibition.

3.4. Biomarker screening between anlotinib responders and non-responders via proteomics analysis

Understand the baseline plasma protein levels between responders and non-responders will contribute to provide potential biomarker screening for anlotinib responsive stratification. Here, we found that 514 common proteins both existed in the plasma samples from responders and non-responders. Of 514 proteins, 23 proteins with higher level and 21 proteins with lower levels from responders, compared with those from non-responders (Fig. 5a). Heat map analysis suggested that majority of those differential proteins stably existed in the duplicated samples (Fig. 5b). Those differential proteins enriched in the

biological process items including peptide cross-linking, regulation of peptidase activity, cornification, and so on (Fig. 5c). Those higher level proteins from responders at baseline enriched in the items of cellular process, metabolic process, single-organism process, and so on. And those lower level proteins enriched in the items of response to stimulus, biological regulation, regulation of biological process, and so on (Fig. 5d). Cell component analysis suggested those differential proteins enriched in extracellular space, extracellular region part, extracellular region, and so on (Fig. 5c). Furthermore, we compared the plasma protein levels between responders and non-responders at the time point of progression disease. Results suggested that there are 4 proteins with higher levels in non-responders than those in responders, and 15 proteins with lower levels in non-responders than those in responders (Supplementary Fig. 2). These results suggested potential biomarkers could be screened out for anlotinib stratification.

3.5. Integrative analysis reveals ARHGDIB levels potentially be used for anlotinib responder screening

To further screen out the potential plasma biomarker, integrative analysis was performed on those protein levels at three time points of baseline, best response, and progression disease from responders, and at the time point of baseline from non-responders. After filtered, we found 43 proteins showed important potential value (Fig. 6a). For the samples from non-responders at the time point of baseline, of 43 proteins, 5 proteins with lower level and 38 proteins with higher level (Fig. 6b). Biological process analysis suggested these proteins enriched in the items of receptor-mediated endocytosis, platelet degranulation, innate immune response, and so on. Cellular component analysis suggested these proteins enriched in the items of protein binding, Poly (A) RNA binding, calcium ion binding, and so on. Molecular function analysis suggested these proteins enriched in the items of extracellular exosome, extracellular region, extracellular space, and so on (Fig. 6c). Among these proteins, we selected the *ARHGDIB* maybe have some predictive value due to anlotinib can down-regulate *ARHGDIB* expression in NCI-H1975 cells, and those patients with lower *ARHGDIB* expression have longer OS outcome in TCGA cohort (Fig. 6d, 6e). Furthermore, we detected the plasma ARHGDIB levels at baseline on those responders and non-responders, and found that high levels of plasma ARHGDIB in NSCLC patients have a better response to anlotinib than those patients with a low level of plasma ARHGDIB [High (n = 14), Median PFS = 189 days versus High (n = 14), Median PFS = 40.5 days, P < 0.001; High (n = 14), Median OS = 375 days versus High (n = 14), Median PFS = 158.5 days, P = 0.034] (Fig. 6f, 6g). These results suggested proteomics analysis potential used for anlotinib responsive stratification.

4. Discussions

Anlotinib as a multi-target TKI inhibitor has been demonstrated the antitumor effectively on different cancers in clinical trials[1, 3–5, 7]. Due to the main mechanism of anti-angiogenesis, the complex architecture of signaling pathway limits the anlotinib biomarker screening[11]. The previous studies have been discussed the potential molecular mechanism and biomarker of anlotinib based on RNA-seq and DNA-seq platform[8, 10, 11, 16]. But for these issues, it is still unclear based on the proteomics platform. In order to understand the underlying value, here we performed proteomics on those plasma samples from different stratification of anlotinib-administrated patients at different time points.

Proteomic technology has been used in biomedical research widely since its development[17–19]. Ying Jiang et al. found that proteomic could identify novel therapeutics targets for hepatocellular carcinoma[20]. Mark A Eckert et al. suggested the metabolic regulator *NNMT* can be screened out via proteomics[21]. Furthermore, cancer biomarker screening based on proteomics has been introduced to clinical translational researches[19, 22, 23]. Due to complex architecture of anlotinib anti-angiogenic signaling pathway, proteomics can provide the platform just because of it can characterize proteins as a whole. In the present study, we firstly compared the change of plasma protein levels in those anlotinib responders between baseline and best response, and found the proteins with significantly increased or decreased may play an important role in anlotinib-induced antitumor effect. Then, we found 8 proteins potentially associated with anlotinib acquired resistance, after comparing plasma protein levels from anlotinib responders at three different time points including baseline, best response, and progression disease. Lastly, our results indicated that 41 proteins may involve in the compensatory effect of anlotinib-induced signaling pathway alteration, after comparing the plasma protein levels from non-responders at baseline and progression disease.

The development of liquid biopsy now is changing the clinical cancer therapeutic practice[19, 24]. Presently, proteomics-based liquid biopsy guided cancer treatment is still in early stage[19]. Studies have shown that the potential value of screen cancer biomarkers from blood via proteomics[19, 25, 26]. Our results suggested anlotinib-induced the alteration of plasma protein levels from responders may be used as alternative biomarkers. Different baseline plasma protein levels between responders and non-responders also have potential predictive values. Importantly, we found 43 proteins via integrative analysis that could have more predictive values for anlotinib clinical stratification. Although the present study found some novelty results via the proteomics for anlotinib molecular mechanism and biomarker screening, the ongoing effects will be performed to solve the deficiencies in the future.

5. Conclusion

In conclusion, our study firstly provided the difference of plasma proteomics between different time points from the NSCLC patients who received anlotinib therapy. These results will give us a novel insight for study the molecular mechanism, acquired resistance, and biomarker screening of anlotinib.

Declarations

Ethics declarations

Ethics approval and consent to participate

Shanghai Chest Hospital Ethics Committees have approved this work, and written informed consents were obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing financial interest.

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Author contributions

Experiments were conceived and designed by B.H. Han and H.M. Wang. Clinical trials were performed by B.H. Han, H. Zhong, W. Zhang, and H.M. Wang. Proteomics experiments were performed by J. Lu, L.L. Zhang, W. Zhang, Y.Q. Lou, W. Nie, and P. Gu. Clinical analysis, bioinformatics analysis and statistical analysis were performed by J. Lu, L.L. Zhang, W. Zhang, X.D. Zhao, Y.Q. Lou, J. Qian, J. Xu, and W. Nie. The manuscript was written by J. Lu, and revised by B.H. Han.

Availability of data and material

All raw data were deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository with the project ID: IPX017919.

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Figures

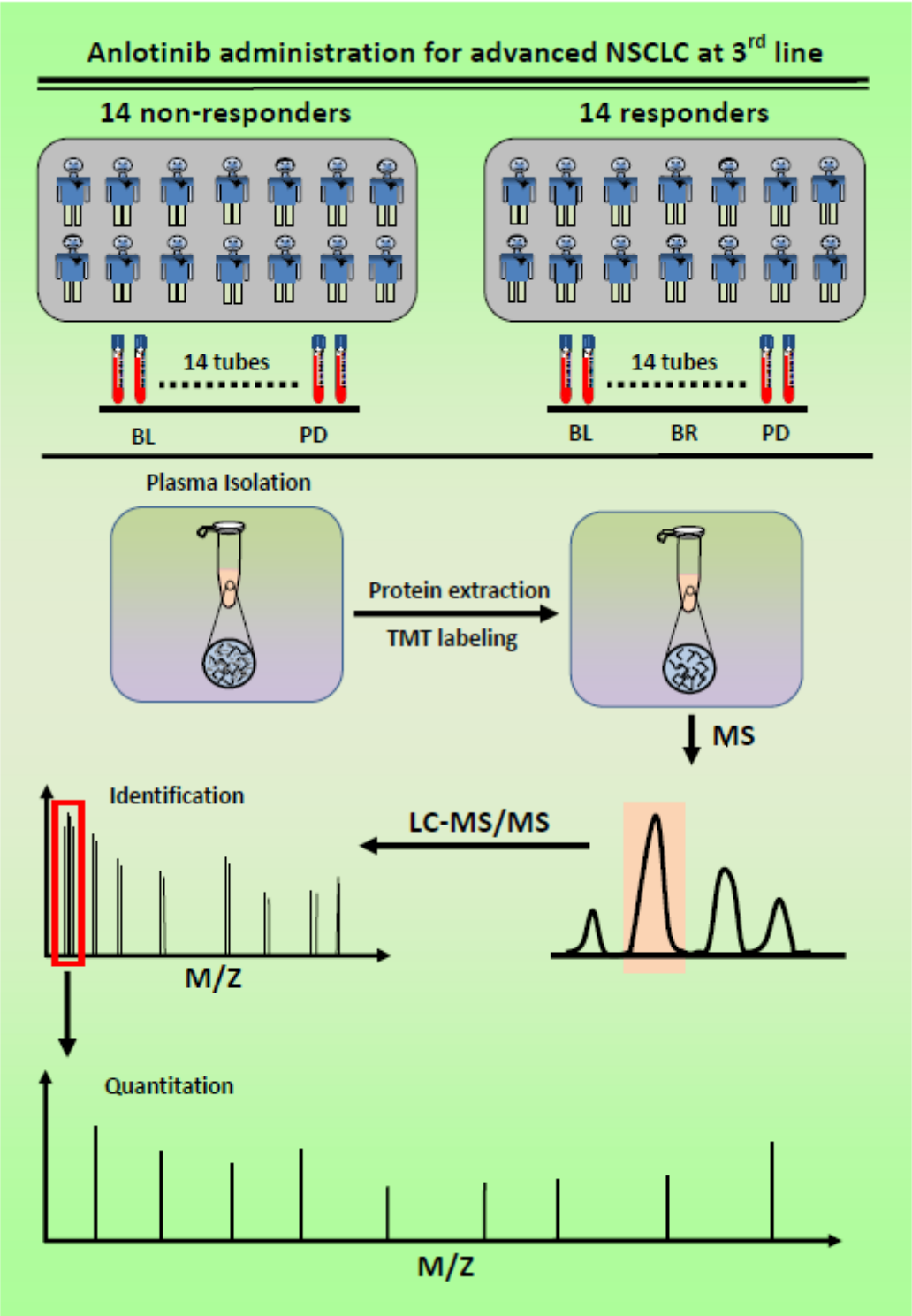

Figure 1

The flowchart of proteomics of plasma samples from NSCLC patients with anlotinib responders and anlotinib non-responders. Plasma samples were performed protein extraction, TMT labelling, LC-MS/MS identification, and quantitation for proteomics analysis.

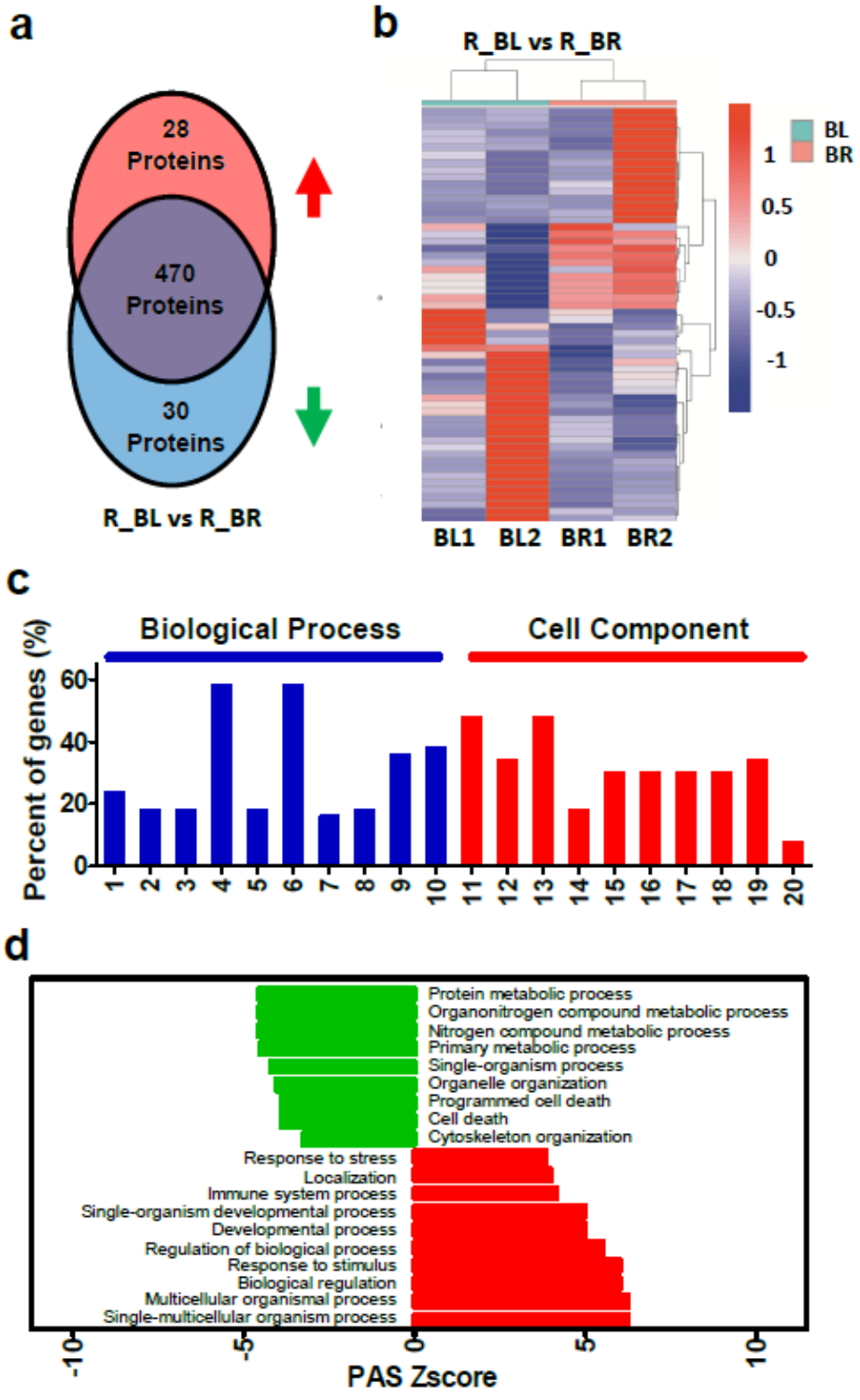

Figure 2

Analysis of the differential protein levels between baseline and best response in anlotinib responders. (a) Venn diagram analysis of the differential proteins that were modulated by anlotinib*. (b) Heat map representation of protein differentially levels between baseline and best response. (c) Biological process and cell component analysis for those differential proteins#. (d) Biological process analysis for those up-regulated and down-regulated proteins respectively. *R_BL: the plasma collected from responders at

baseline. R_BR: the plasma collected from responders at best response. #1. Inflammatory response; 2. Keratinization; 3. Acute inflammatory response; 4. Single-multicellular organism process; 5. Keratinocyte differentiation; 6. Multi-cellular organismal process; 7. Cornification; 8. Epidermal cell differentiation; 9. Immune response; 10. Immune system process; 11. Extracellular region part; 12. Extracellular space; 13. Extracellular region; 14. Intermediate filament cytoskeleton; 15. Extracellular membrane-bounded organelle; 16. Extracellular exosome; 17. Extracellular vesicle; 18. Extracellular organelle; 19. Membrane-bounded vesicle; 20. Cornified envelope.

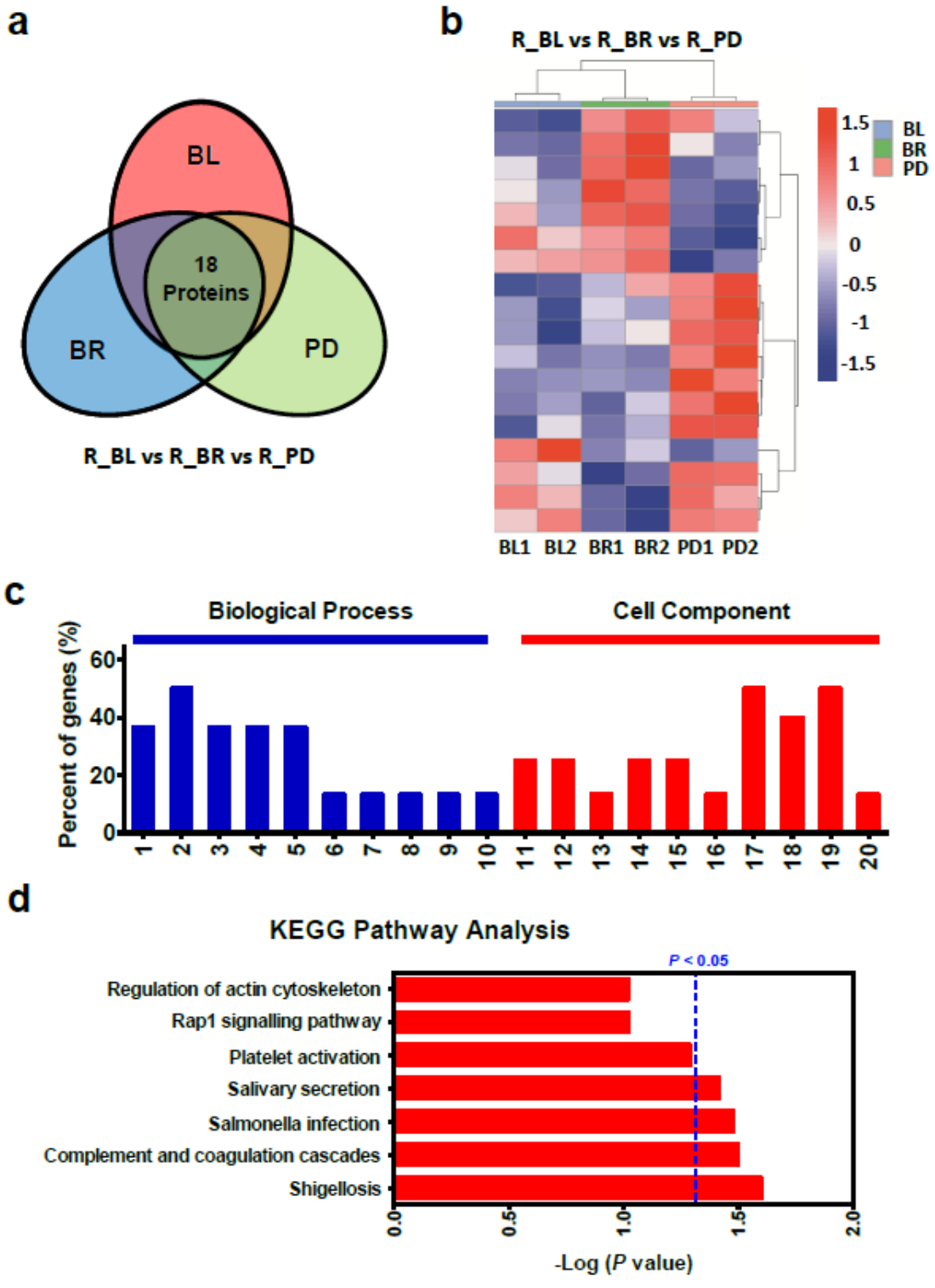

Figure 3

Integrative analysis of differential protein levels between baseline, best response, and progression disease in anlotinib responders. (a) Venn diagram analysis of the differential proteins*. (b) Heat map representation of protein differentially levels between baseline, best response and progression disease. (c) Biological process and cell component analysis for those differential proteins#. (d) KEGG pathways analysis of 18 differential proteins. *R_BL: the plasma collected from responders at baseline. R_BR: the plasma collected from responders at best response. R_PD: the plasma collected from responders at progression disease. #1. Platelet activation; 2. Cell activation; 3. Blood coagulation; 4. Hemostasis; 5. Coagulation; 6. Cellular response to granulocyte colony-stimulating; 7. Negative regulation of blood coagulation; 8. Regulation of blood coagulation; 9. Blood coagulation, common pathway; 10. Response to granulocyte colony-stimulating factor; 11. Platele alpha granule; 12. Blood microparticle; 13. Mucus layer; 14. Cell cortex; 15. Cytoplasmic region; 16. Fibrinogen complex; 17. Membrane-bounded vesicle; 18. Extracellular space; 19. Vesicle; 20. Secretory granule.

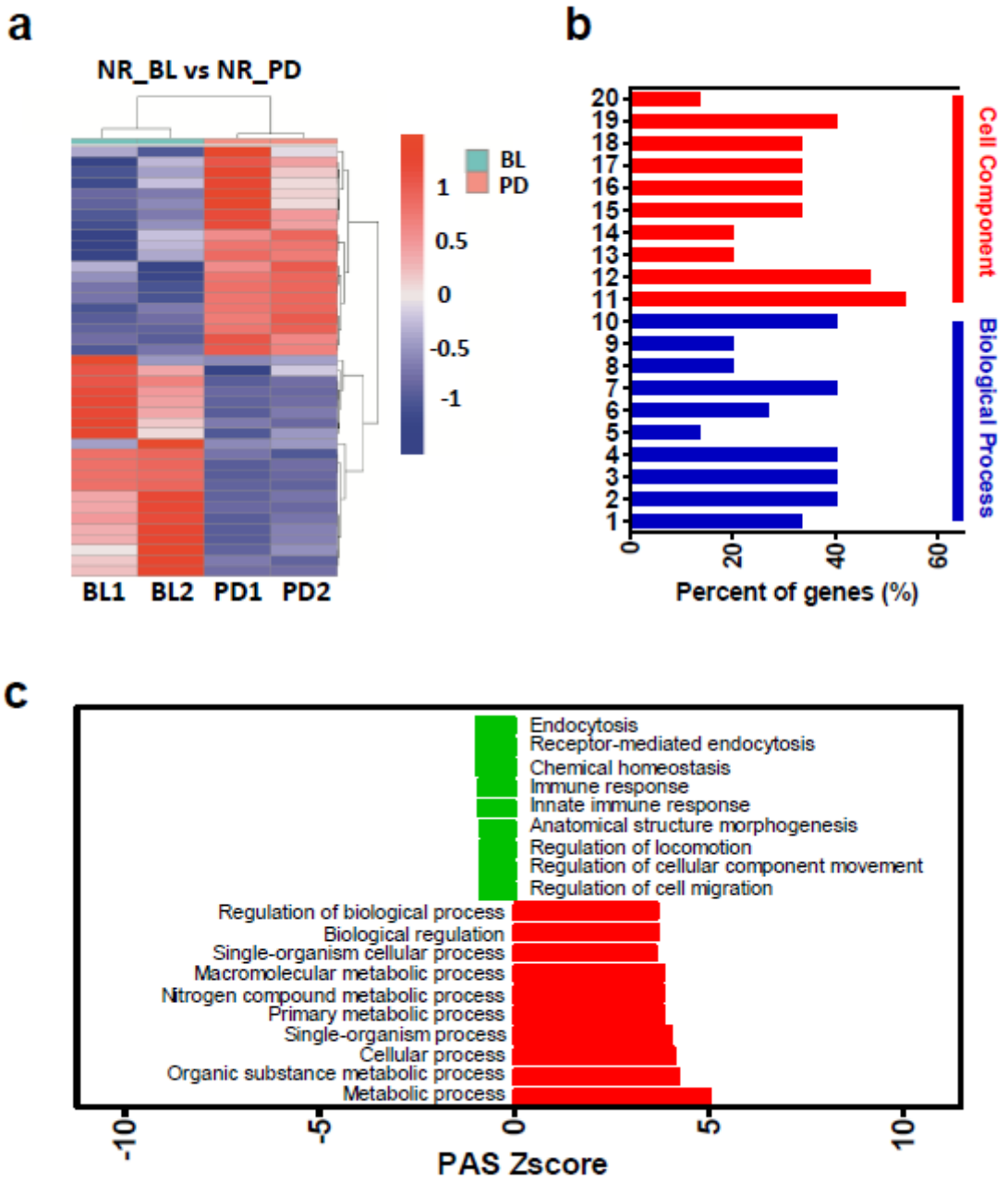

Figure 4

Analysis of the differential protein levels between baseline and best response in anlotinib non-responders. (a) Venn diagram analysis of the differential proteins that were modulated by anlotinib*. (b) Biological process and cell component analysis for those differential proteins#. (c) Biological process analysis for those up-regulated and down-regulated proteins respectively. *NR_BL: the plasma collected from non-responders at baseline. NR_PD: the plasma collected from non-responders at progression disease. #1. Inflammatory response; 2. Cell migration; 3. Localization of cell; 4. Cell motility; 5. Keratinocyte migration; 6. Leukocyte migration; 7. Locomotion; 8. Cytokine secretion; 9. Acute inflammatory response; 10. Movement of cell; 11. Extracellular region; 12. Extracellular region part; 13. Cytoplasmic membrane-

bounded vesicle lumen; 14. Vesicle lumen; 15. Extracellular space; 16. Extracellular exosome; 17. Extracellular vesicle; 18. Extracellular organelle; 19. Vesicle; 20. Blood microparticle.

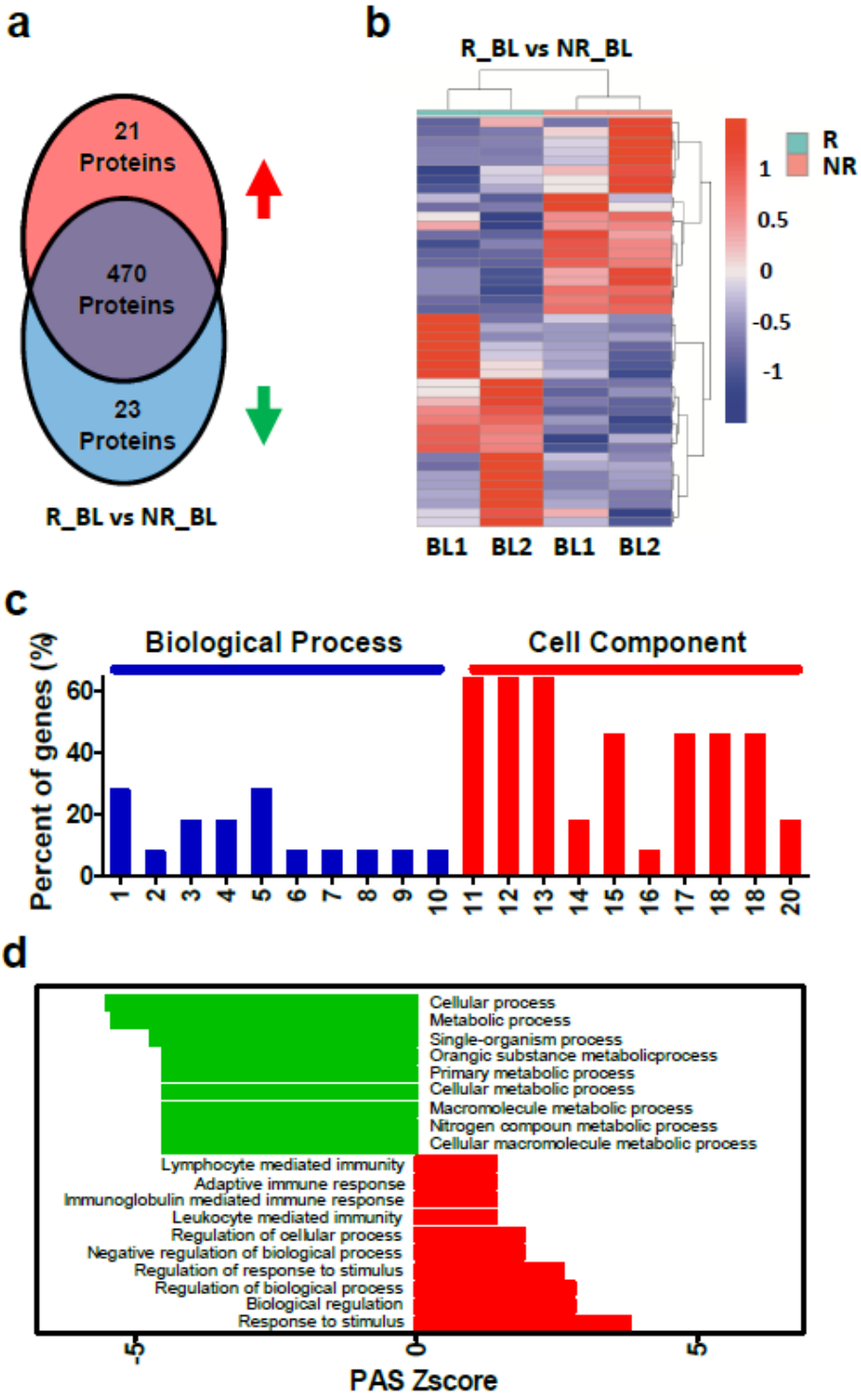

Figure 5

Analysis of the differential protein levels between responders and non-responders at baseline. (a) Venn diagram analysis of the differential proteins*. (b) Heat map representation of protein differentially levels between responders and non-responders at baseline. (c) Biological process and cell component analysis

for those differential proteins#. (d) Biological process analysis for those up-regulated and down-regulated proteins respectively. *R_BL: the plasma collected from responders at baseline. NR_BL: the plasma collected from non-responders at baseline. #1. Peptide cross-linking; 2. Keratinocyte activation; 3. Skin epidermis development; 4. Cornification; 5. Regulation of peptidase activity; 6. Positive regulation of substrate-dependent cell; 7. Regulation of substrate-dependent cell migration; 8. Interaction with other organism; 9. Negative regulation of transforming growth; 10. Calcium-independent cell-matrix adhesion; 11. Extracellular space; 12. Extracellular region part; 13. Extracellular region; 14. Cornified envelope; 15. Extracellular membrane-bounded organelle; 16. Insulin-like growth factor binary complex; 17. Extracellular exosome; 18. Extracellular vesicle; 19. Extracellular organelle; 20. Keratin filament.

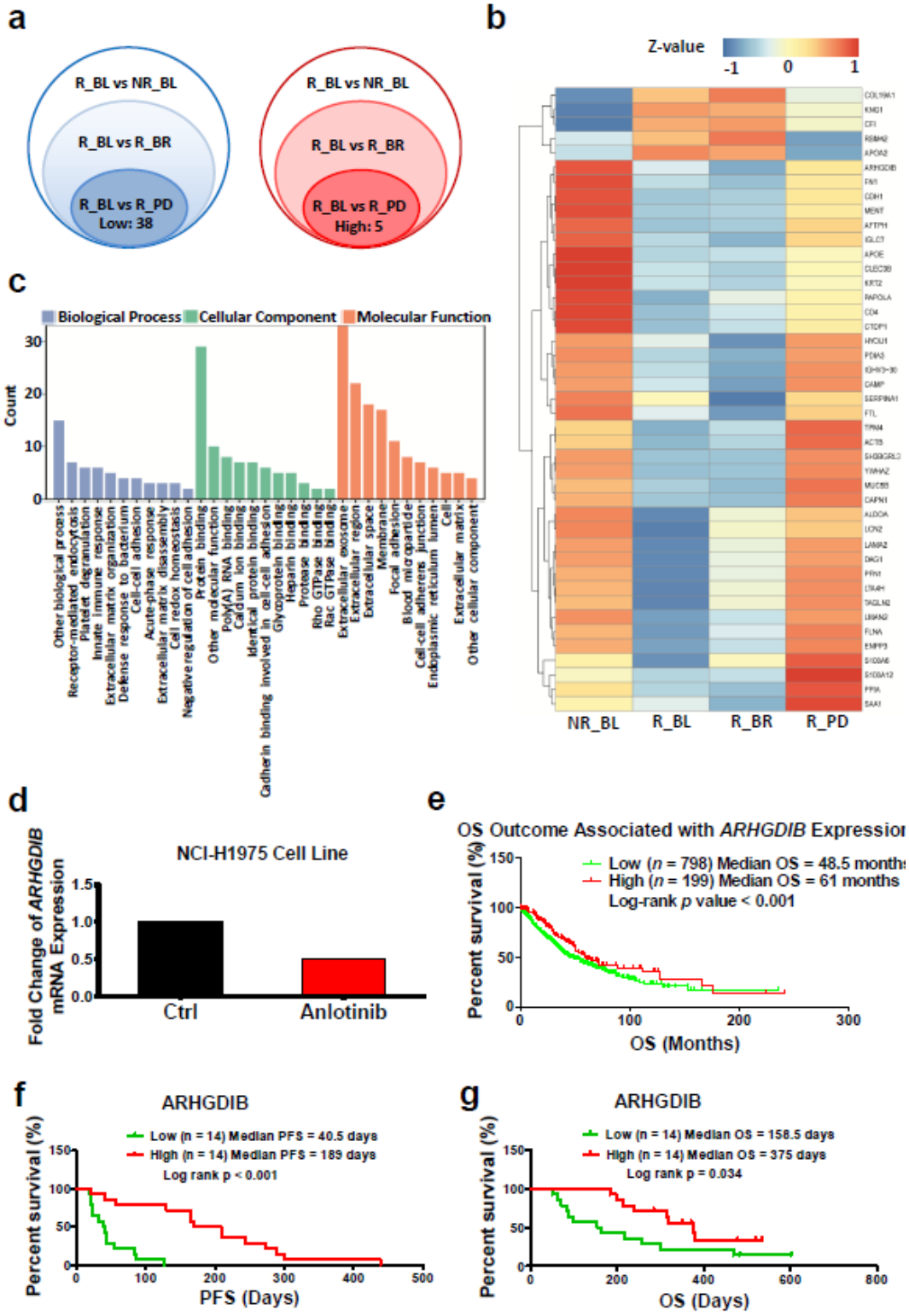

Figure 6

Integrative analysis of plasma protein levels between responders and non-responders at different time points. (a) Venn diagram analysis of the differential proteins*. (b) Heat map representation of protein differentially levels between responders and non-responders at different time points. (c) Analysis biological process, cell component and molecular function for those differential proteins. (d) NCI -H1975 cells were exposed to anlotinib (8 μg/ml) for 24 hours. mRNA expression of ARHGDIB was detected by

RNA-seq. (e) Kaplan-Meier plots of overall survival in NSCLC patients from TCGA cohort based on ARHGDIB expression stratification. (f, g) Kaplan-Meier curves of PFS and OS via stratifying the plasma ARHGDIB levels in advanced refractory NSCLC patients treated with anlotinib. n = 28, Cutoff-High: 14 patients, Cutoff-Low: 14 patients. * R_BL: the plasma collected from responders at baseline. R_BR: the plasma collected from responders at best response. R_PD: the plasma collected from responders at progression disease. NR_BL: the plasma collected from non-responders at baseline.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.pdf