Serum cholesterol-riched apolipoprotein-B (apoB) containing lipoproteins predict the response to immune checkpoints inhibitors (ICIs) in non-small cell lung cancer (NSCLC) : A retrospective analysis

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Abstract

Background

Lipid metabolism alternations reprogrammed tumor microenvironment (TME) to participate in clinical response to immune checkpoint inhibitors (ICIs) in non-small cell lung cancer (NSCLC). Body mass index (BMI), clinically used to assess body fat, has been positively correlated with ICIs monotherapy response. Circulating cholesterol is mainly packaged into apolipoproteins B (apoB) in the form of apoB containing lipoproteins, including low-density lipoprotein cholesterol (LDL-C) and remnant cholesterol (RC), which have been regarded as risk factors of tumorigenesis. The association between BMI, lipoproteins, and clinical outcome of ICIs is far from clear.

Methods

We performed a retrospective case-control analysis of 99 NSCLC patients treated with chemotherapy plus ICIs regimens in the department of oncology and lung cancer center of China-Japan Friendship Hospital between January 2019 to December 2021 and assessed the prognostic value of the BMI, serum lipids, lipoproteins, and apolipoproteins. Efficacy was assessed by response evaluation criteria in solid tumors (RECIST) version 1.1 and evaluated by the ICIs response (durable clinical benefit [DCB] / non-durable benefit [NDB]), best response (active/ non-active) and progression-free survival (PFS). The validation test and the mechanism exploration were performed with public data using bioinformatic methods.

Results

The contrary results to the ICIs single agents were obtained. BMI $\geq$ 25 kg/m^2 was a risk indicator for NDB, non-active response to ICIs, shorter PFS with the confounders adjusted (OR = 6.06, 95% CI 2.05–19.89, p.val = 0.002; OR = 4.37, 95% CI 1.64–12.43, p.val = 0.004; HR = 3.08, 95% CI 1.63–5.82, p.val < 0.001). High cholesterol-riched apoB containing lipoproteins were risk factors for poor ICIs response. Low RC predicted the better ICIs response and the best response with the satisfied discriminative ability and the well-fitting calibration curves (OR = 0.12, 95%CI 0.02–0.63, p.val = 0.017; OR = 0.22. 95%CI 0.05–0.96, p.val = 0.047). Serum LDL-C predicted the longer PFS of ICIs with the added value to the BMI containing model (HR = 0.43, 95%CI 0.22–0.86, p.val = 0.016). Bioinformatic exploration in the TCGA-LUAD cohort showed that the APOB high group was infiltrated by elevated fibroblasts and other immune-suppressive cells to acquire the stromal barrier and form non-inflamed TME. Functional enrichment analysis indicated that the acquired ICIs resistance might be through the LRP6/5-Wnt-TGF-β axis which required laboratory experiments to verify.

Conclusions

In the NSCLC population treated with the combination of chemotherapy and ICIs, BMI ≥ 25 kg/m^2, the elevated cholesterol-riched apoB containing lipoproteins are promise indicators for the poor ICIs response. Further large-scale validations and experimental explorations are needed.

1 Background

Lung cancer accounts for the leading cause of cancer-associated mortality, about 85% of which are non-small cell lung cancers (NSCLC) including squamous cell carcinoma, adenocarcinoma, and large cell carcinomas ([1]). Immune checkpoint inhibitors (ICIs) targeting the programmed cell death protein 1 (PD-1)/ programmed death-ligand 1 (PD-L1) axis have markedly led to a dramatic change in the treatment of NSCLC especially those without molecular targets. Chemotherapy can enhance tumor antigenicity to improve the efficacy of ICIs and lower the incidence of immune-related adverse events (irAEs). Thereby, the combination regimen of chemotherapy and ICIs has been considered the standard first-line treatment for NSCLC patients without sensitive mutations ([2]).

Lipids participate in energy source and storage and play critical roles in the constitution of the structural basis of biological membranes and signaling molecules. Altered lipid metabolism is among the most prominent metabolic alterations in cancer. Enhanced synthesis or uptake of lipids contributes to tumorigenesis ([3]). The crosstalk between altered lipid metabolisms and the adaptable tumor microenvironment (TME) can strongly reprogram tumor initiation, growth, invasion, metastasis, and response to therapies. Lipid metabolism was also plastic and shaped by TME, which allows tumors to thrive ([4], [5]).

Obesity-associated inflammation dysregulates immune response, potentially leading profound effects on the toxicity and efficacy of ICIs. Body mass index (BMI) is clinically used to assess body fat in human subjects ([6], [7]). Past studies declared a positive prognostic role of high BMI in NSCLC patients treated by first-line single agent ICIs-based regimens, whereas conflict result was obtained in the chemoimmunotherapy combinations (6-[8]). Consistently, high BMI was demonstrated to be associated with high occurrence of immune-related adverse events (irAEs) ([9], [10]).

Circulating lipids including cholesterol and triglycerides are packaged into apolipoproteins and transported in the form of lipoproteins. Lipoproteins are classified by their density and in a state of continuous flux due to the shifting of the composition of lipids within the lipoproteins in the blood circulation ([11]). The cholesterol carried by apolipoprotein B (apoB) containing lipoproteins, including low-density lipoprotein cholesterol (LDL-C) and remnant cholesterol (RC) are the predominant atherogenic agent that feeds the development of arterial wall plaques ([12]). Researchers revealed that statins showed a positive impact on the ICIs treatment outcome ([13]). The positive association of cancer risk and apoB level has been declared, while high LDL-C was suggested to be associated with high incidence of cancer and metastasis in most cancers ([14], [15]). Recent studies indicated a positive correlation between LDL-C, HDL-C and serum cholesterol and the ICIs monotherapy response, whereas the contrary results were

obtained in the chemotherapy groups ([16]). Since the apoB containing lipoproteins have been shown as the risk factors for tumorigenesis, the results remained to be controversial ([17]). In the light of the available evidence, the roles of lipoproteins in the ICIs response are far from clear.

To elucidate the association between lipoproteins and clinical outcome of ICIs in NSCLC, we performed a retrospective case-control analysis of NSCLC patients treated with chemotherapy plus ICIs regimens in the department of oncology and lung cancer center of China-Japan Friendship Hospital.

2 Materials And Methods

2.1 Patient cohorts:

We retrospectively collected the clinical data of NSCLC who were administrated with the regimens of chemotherapy plus anti-PD-1/PD-L1 from January 2019 to December 2021 and followed up in the department of oncology and lung cancer center at China-Japan Friendship Hospital. For the inclusion criteria: **(1)** Patients had a diagnosis of Stage III-IV NSCLC at the first ICIs initiation regardless of the treatment line. The radiographic data or medical records used to evaluate the efficacy of ICIs were required. The disease was staged according to the 7th and 8th editions of the tumor, node, metastasis system (TNM) classification ([18]). **(2)** Patients with Stage I - II NSCLC who pre-operatively received ICIs and had the post-operative pathological data used to confirm the efficacy of the neoadjuvant therapy. For the exclusion criteria: **(1)** Patients whose lung or mediastinal tumor lesions received radiotherapy or interventional ablation therapy during the ICIs treatment. **(2)** Patients who were not evaluable radiologically. **(3)** Patients discontinued ICIs for the detected sensible Epidermal Growth Factor Receptor (EGFR) alterations to receive target agents.

The clinical response was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 ([19]). Computed tomography (CT) studies were independently read by two radiologists. The primary outcome measured was progressive disease (PD). Progression-free survival (PFS) was assessed from the start date of chemoimmunotherapy to the date of progression. Patients who discontinued ICIs for the serious adverse events or had not progressed at the termination of data collection were censored at the date of their last scan. Cases retrospectively adjudicated to not be PD per RECIST but determined in real-time by the treating clinician as PD were considered as events. In the study, the evaluation was stable disease (SD) with insufficient shrinkage to qualify for PR, then the response was considered as SD-a. SD-b was defined as SD without sufficient increase to qualify for PD. If the target lesion had no changes radiologically, the response was determined as SD.

The clinical response to ICIs was defined with two methods. Firstly, durable clinical benefit (DCB) was defined as partial response (PR) or SD that lasted more than 6 months, whereas non-durable benefit (NDB) was considered as progressive disease (PD) or SD that lasted less than 6 months ([20]). Secondly, the clinical response was also evaluated as the best response to ICIs. The best response to ICIs was PR or SD-a was regarded as the active response to ICIs. Patients with SD lasting more than 6 months were also

classified as active in ICIs. The non-active response to ICIs was defined by meeting any of the following conditions: (1) The evaluation of PD in the first following-up; (2) The best response to ICIs was SD-b, indicating the disease progressed slowly during the treatment of ICIs; (3) SD lasted less than 6 months.

The ICIs response (NDB/DCB) was regarded as the primary outcome. The secondary outcome was the best response to ICIs and the incidence of irAEs of any grade. IrAEs were graded according to the US National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE; version 5.0).

We complied with all relevant ethical regulations for working with human participants. Informed consent was obtained. This study was approved by the institutional review board at China-Japan Friendship Hospital.

2.2 Collection of clinical data

Baseline demographic and clinical data were collected from patients' medical records on the first administration of ICIs or during the ICIs treatment. Self-reported cigarette smoking and physical activity characteristics were extracted from the medical records. Diabetes status (yes/no) at baseline was based on either one of the following criteria: fasting plasma glucose levels greater than 126 mg/dL, non-fasting plasma glucose >200 mg/dL, glycated hemoglobin (HbA1c) $\geq$ 6.5%, taking anti-diabetic medication within 2 weeks of baseline data collection, or self-report of a diagnosis of diabetes. Smoking history (yes/no) was defined as either active smoking or having a previous smoking history. The patients' pre-treatment height and body weight were recorded. BMI was calculated by dividing a patient's weight in kilograms (kg) by the square of the patient's height in meters (m). BMI was classified according to the World Health Organization (WHO) categories: underweight, BMI < 18.5 kg/m^2; normal, 18.5 kg/m^2 $\leq$ BMI $\leq$ 24.9 kg/m^2; overweight, 25 kg/m^2 $\leq$ BMI $\leq$ 29.9 kg/m^2; obesity, BMI $\geq$ 30 kg/m^2.

Blood samples were obtained after 8-hour of overnight fasting before the pre-treatment or during the treatment of ICIs. Serum concentrations of total cholesterol (TC) (mmol/L), total triglycerides (TG) (mmol/ L), high-density lipoprotein cholesterol (HDL-C) (mmol/L), LDL-C (mmol/L), apoA1 (g/L), apoB (g/L) levels were measured and recorded. The baseline apoB/(apoB+ApoA1) ratio was calculated as apoB level divided by the sum of apoB and apoA1 levels. RC is the cholesterol content of all non-LDL-C and non-HDL-C and was calculated as TC minus LDL-C and HDL-C. Patients were divided into two groups based on the threshold of serum LDL-C and RC levels. The thresholds of LDL-C and RC were selected by the "surv-cutpoint" function implemented in the survminer R package since non-reports about the cut points of the markers in NSCLC were available.

2.3 Bioinformatic validation

Public data including GSE126044 and GSE135222 were downloaded from Gene Expression Omnibus (GEO) datasets (https://www.ncbi.nlm.nih.gov/) used for the validation of the correlation between apoB

level and the ICIs response ([21], [22]). The Cancer Genome Atlas (TCGA) lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) cohorts' level 3 RNA-seq data (HTSeq-Counts) were directly downloaded by GDC data transfer tool (https://portal.gdc.cancer.gov/). Minus germ-line somatic copy number alternations (sCNA) and merged somatic simple nucleotide variations (sSNV) segmented data of TCGA LUAD cohort were downloaded from GDAC Firehose (Broad Institute TCGA Genome Data Analysis Center, https://gdac.broadinstitute.org/) for oncogene status analysis. The corresponding DNA methylation data (IDATs) of TCGA-LUAD and TCGA-LUSC methylation beta value was obtained. The Illumina Human Methylation450 bead chip raw data were processed by minfi and ChAMP package ([23], [24]).

LUAD samples with EGFR mutations were excluded from the analysis for the acknowledged resistance to ICIs. The immunophenotypes of TCGA NSCLC samples were classified with the method previously introduced by single-sample gene set enrichment analysis (ssGSEA) ([25], [26]). The immunologically cold tumors, which were characterized by fewer infiltrated immune cytotoxic cells and low expression of immune regulators in the tumor microenvironment (TME), were excluded from the analysis for the insensitivity to the ICIs. The consistency of different clusters was verified by cytolytic activity (CYT), which represented the ultimate effective mechanism in the cancer immunity cycle and was calculated as the geometric mean of granzyme A (GZMA) and perforin (PRF1) expression levels as previously defined ([27]). Another two methods were applied to measure the TME, including MCPcounter and EpiDISH, which were used to estimate the population abundance of tissue-infiltrating immune and stromal cell populations in heterogeneous tissues from transcriptomic data and methylation data respectively ([28], [29]). The validation cohorts were divided into two groups respectively according to the median expression of APOB genes of every cohort. Functional enrichment analysis between groups was realized by GSVA based on gene expression data matrices with ([30]). Differentially expressed genes (DEGs) were identified by DESeq2 between NDB and DCB patients in the LUAD subgroup of GSE126044 and among the immunophenotype clusters in the TCGA-LUAD cohort ([31]).

2.4 Statistical analysis

Statistical analysis was conducted with R software (version 4, 4.0.4). Baseline characteristics are presented as means and standard deviations, or as medians and interquartile ranges (IQR) for continuous variables, and as frequencies and percentages for categorical variables. BMI was regarded as a continuous and categorical variable respectively. Patients with BMI $\geq$ 25mg/m^2 were combined as the overweight and obesity group for analysis and BMI < 25 kg/m^2 was treated as the reference. T-test or Mann Whitney U test was used for comparison between the two groups. Distributions of the groups were estimated by the Chi test. The unadjusted univariable and multivariable logistic regression models were used to estimate crude and adjusted odds ratios (OR) for BMI, circulating apolipoprotein, and lipids respectively. BMI was also regarded as a confounder to influence the serum lipids. The other confounders including age, gender (female or male), DM (yes or no), and smoking history (yes or no) were used for the

multivariate regression. Time-of-follow-up was calculated from the date of the first initiation of ICIs to the date of the PD, ICIs discontinuation for the severe AE, or end of follow-up (May 1, 2022). Cox proportional hazards models were applied for the analyses of circulating apolipoprotein and lipids and the duration of ICIs and to compute the hazard ratios (HRs) with 95% CIs. Multivariate analyses were adjusted for potential confounders mentioned above. PFS was evaluated and compared using the Kaplan-Meier (KM) method and the log-rank test for trends. Receiver operating characteristic (ROC) curves were used to evaluate the discriminative ability of the markers by pROC package. The time-dependent ROC curves at 3-year of the Cox model were pictured by the timeROC R package ([32]). The efficiency of models was assessed by comparing the decision curve analyses (DCA) curves of models ([33]). All p values were two-sided, and confidence intervals (CIs) were set at the 95% level, with significance predefined to be at < 0.05.

3 Results

3.1 Patients' characteristics

The workflow of this study was showed in **Figure 1**. From January 2019 to December 2021, a total of 102 patients with NSCLC treated with combination of chemotherapy and ICIs in China-Japan Friendship Hospital were enrolled in this study. 3 patients didn't complete the first evaluation for the first administration of ICIs starting recently. Therefore, a total of 99 patients were enrolled for analysis. Patient baseline clinical characteristics are summarized in **Table 1**. Of these, there were 55 patients (55.6%) with LUAD, 37 patients (37.4%) with LUSC, the others were large cell carcinomas or mixed NSCLC. The median age was 64 years (58 - 69). 15 patients (15.2%) were female. 4 patients (4.04%) suffered from Stage I to IIIa disease and received ICIs as pre-operative adjuvant therapy. The 99 patients were all in good condition (the range of Eastern Cooperative Oncology Group Performance Status [ECOG-PS] was 0–1 score). 18 patients (18.2%) with LUAD had the recorded KRAS mutations. 58 patients (58.6%) were normal weight, 36 patients (36.4%) were overweight, and only 5 patients (5.05%) were obese. No patients were underweight. The overweight and obese groups were thereby combined as the BMI $\geq$ 25kg/m^2 group. No significant difference between the BMI < 25kg/m^2 and the BMI $\geq$ 25kg/m^2 group in the baseline circulating lipids, lipoproteins, and apolipoproteins (**Table S1**). The mean follow-up period for this study was 9.56 months. 75 patients which had been followed up for more than 6 months and had the primary endpoint (DCB or NDB). The best response to ICIs (non-active or active) of 89 patients was available. irAEs of 99 patients were recorded. Patient demographics and disease characteristics including age, gender, smoking history, and diabetes status were generally balanced between the NDB and DCB arms (**Table S2**), what's more, between the non-active and active response arms (**Table S3**).

3.2 BMI $\geq$ 25 kg/m^2 predicts poor ICIs response in NSCLC

Comparations between groups divided according to the duration of responding to ICIs (NDB or DCB) and best response to ICIs (non-active or active) were performed. NDB patients were more likely to have higher

BMI (T-test, 25.71 kg/m^2 vs 23.29 kg/m^2, p.val = 0.034, **Figure 2A**). The prevalence of BMI was higher in the cases with the non-active response, but the difference was not statistically significant (T-test, 25.64 kg/m^2 vs 24.40 kg/m^2, p.val = 0.065, **Figure 2B**).

BMI ≥ 25 kg/m^2 was a negative predictor for DCB and active response to ICIs. The OR for NDB was 6.06 (95% CI 2.05 - 19.89, p.val = 0.002) for patients with BMI ≥ 25 kg/m^2 by the adjusted for the confounders including age, gender, diabetes, and smoking history. The OR for non-active response to ICIs was 4.37 (95% CI 1.64 - 12.43, p.val = 0.004) for patients with BMI ≥ 25 kg/m^2 with the confounders adjusted. The area under the ROC curve (AUC) was 0.72 (95% CI 0.60 - 0.85, **Figure 2C**) for the model used to predict DCB. AUC was 0.70 (95% CI 0.57 - 0.83, **Figure 2D**) for predicting active response to ICIs. The calibration curves showed that the multivariate models containing the BMI group tended to underestimate the probability of NDB and non-active response (**Figure 2E-F**).

Patients with normal weight tended to have a longer duration of ICIs response (Log-rank test, median PFS: 17.8 months vs 4.83 months, p.val = 0.001, **Figure 2G**). The HR for PFS of BMI ≥ 25 kg/m^2 without confounding factors adjusted was 2.54 (95% CI 1.41 - 4.60, p.val = 0.002), the HR was 3.08 (95% CI 1.63 - 5.82, p.val < 0.001) by multivariate Cox model, indicating that BMI ≥ 25 kg/m^2 was a negative predictor for the duration of ICIs response. The C-index of the multivariate Cox model was 0.68 (95% CI 0.61 - 0.76). The 3-year AUC was 0.76 and the calibration curve was shown in **Figure 2H-I**.

The subgroup analyses showed that BMI ≥ 25 kg/m^2 was an active indicator of DCB and active response to ICIs according to the univariate logistic regression in all subpopulations (**Figure S1-2**). What's more, patients with BMI ≥ 25 kg/m^2 tended to have a shorter PFS of ICIs in all subgroups (**Figure S3**).

3.3 High levels of remnant cholesterol predict poor ICIs response in NSCLC

Compared with DCB patients, the prevalence of serum cholesterol, RC, and LDL-C were higher in NDB patients, but the difference was statistically significant in RC only (T-test, 0.64 mmol/L vs 0.49 mmol/L, p.val = 0.047, **Table S2**). Serum cholesterol, triglyceride, phospholipid, and HDL-C had no difference between NDB and DCB patients. Circulating apolipoproteins including apoA1, apoE, apoC2, and apoC3 did not significantly differ between patients with NDB and DCB. Circulating lipids, lipoproteins, and apolipoproteins had no significant difference in the active and non-active groups base on the best response to ICIs in the total NSCLC cohort (**Table S3**).

Based on the PFS of ICIs, the total group was divided into RC high group and RC low group according to the serum RC levels. Similarly, the total cohort was also grouped by LDL-C level and apoB level. The cut points of RC and LDL-C were selected as 0.82 mmol/L and 2.58 mmol/L respectively. The analogous analyses of the independent and joint effects of apoB containing lipoproteins cholesterol (RC and LDL-C) and apoB levels and BMI were performed. Both continuous and categorical for of each marker were

respectively brought into univariate and multivariate logistic regression model with the confounders including age, gender, diabetes, smoking history, and BMI adjusted.

The OR of RC for NDB and the non-active response was shown in **Table 2**, indicating that high serum RC potentially promoted ICIs resistance which led to NDB and non-active response to ICIs. The AUC of the multivariate model containing RC level (continuous) was 0.78 (95% CI 0.67 - 0.89, **Figure 3A**) for predicting DCB and 0.74 (95%CI 0.63 - 0.86, **Figure 3B**) for predicting active response to ICIs. The AUC of the model containing low RC was 0.79 (95%CI 0.68 - 0.91, **Figure 3C**) and 0.75 (95%CI 0.63 - 0.86, **Figure 3D**) respectively. The calibration curves showed that the multivariate models RC level and low RC all presented satisfied coherence in predicting NDB probability (**Figure 3E-F**). When it comes to predicting non-active response, the models tended to overestimate the rates when the incidence of non-active response was low and underestimate the rates when the incidence was high (**Figure 3G-H**).

The OR of low LDL-C for NDB calculated by univariate logist model was 0.31 (95% CI 0.10 - 0.89, p.val = 0.036), however the OR with the confounders including age, gender, diabetes, smoking history, and BMI adjusted was not statistically significant (OR = 0.32, 95%CI 0.09 - 1.03, p.val = 0.065). Low serum LDL-C was a protective indicator for active ICIs response both without and with the confounders adjusted (OR = 0.29, 95%CI 0.09 - 0.81, p.val = 0.026; OR = 0.24, 95%CI 0.06 - 0.77, p.val = 0.023). The AUC was 0.76 (95%CI 0.66 - 0.86), but the calibration ability was unsatisfied (**Figure S4A-B**).

3.4 Low serum LDL-C predicts long PFS of ICIs in NSCLC

Patients with high RC and LDL-C were more like to respond to ICIs with shorter PFS (Log-rank test, median PFS: 14.53 months vs 3.23 months, p.val = 0.005; median PFS: 16.00 months vs 7.40 months, p.val = 0.010; **Figure 4A-B**). Considering BMI was a potentially important confounding factor for ICIs response and serum apoB containing lipoproteins level, the multivariate Cox regression of RC and LDL-C was performed (**Table S4**). Low LDL-C was a statistically significant protective factor for the long PFS with the BMI and other confounders adjusted (HR = 0.43, 95%CI 0.22 - 0.86, p.val = 0.016). The serum LDL-C level in continuous form was a negative indicator for ICIs PFS (HR = 1.50, 95%CI 1.01 - 2.24, p.val = 0.045). AUC of the multivariate model with LDL-C (continuous and category) at 3-year was 0.76 and 0.79 respectively (**Figure 4C-D**). The calibration curves were pictured in **Figure 4E-F**. The comparison of Cox models used to predict PFS was performed. Compared with only including the BMI category, the adding of serum LDL-C or LDL-C group was more efficient (Anova-test, p.val = 0.049, p.val < 2e-16). The DCA curves showed that the model with the LDL-C group was superior to the model with serum LDL-C in the continuous form and the model without LDL-C (**Figure 4G**). Serum RC and RC category did not add the extra value to the multivariate Cox model with the BMI category.

Subgroup analysis was performed according to the BMI group. Patients with low RC and LDL-C showed a trend to respond to ICIs with longer PFS in both groups but were statistically different in the BMI < 25 kg/m^2 group (**Figure 5A-E**). The multivariate Cox models of RC and LDL-C (continuous and category) were performed by adjusting confounders including gender, age, diabetes status, and smoking history.

Low levels of LDL-C and RC were protective factors in the BMI < 25 kg/m^2 subgroup but not in the BMI ≥ 25 kg/m^2 group (**Figure 5F**).

3.5 ApoB containing lipoproteins predict better response to ICIs in LUAD cohort

In the LUAD cohort, the NDB group seemed to have higher serum cholesterol, LDL-C, and RC without a statistical difference (**Figure S5A-C**). Patients with non-active response to ICIs had higher serum LDL-C and CHO (T-test, 3.45 mmol/L vs 2.84 mmol/L, p.val = 0.010; 5.38 mmol/L vs 4.46 mmol/L, p.val = 0.017, **Figure 6A-B**). The prevalence of RC tended to be higher in the non-active group but was not statistically significant (**Figure 6C**). The univariate and the multivariate models including RC (both continuous and categorical), and LDL-C (both continuous and categorical) in the LUAD subgroup demonstrated that low RC and low LDL-C were protective factors for active ICIs response (**Figure 6D**). However, similar analyses were performed in the LUSC subgroup, serum apoB containing lipoproteins did not show satisfactory ability in predicting the ICIs response (**Table S5**).

Patients with low LDL-C and low RC tended to respond to ICIs with longer PFS (**Figure 6E-F**). The Kaplan-Meier curves of the LDL-C and RC groups were crossed in the LUSC cohort (**Figure S5D-E**). The univariate Cox analysis diagnosed the low LDL-C and low RC as protective factors for long PFS (**Figure 6E-F**), but only the HR of low LDL-C was statistically significant with the confounders adjusted (HR = 0.34, 95%CI 0.12 - 0.99, p.val= 0.047).

3.6 Correlation between serum lipids, lipoproteins and apolipoproteins and ICIs related adverse events (irAEs)

Until May 1st, 2022, 38/99 patients (38.4%) occurred irAEs, 9 patients stopped ICIs for irAEs. Patients were grouped according to the occurrence of irAEs. Difference of BMI between the two groups were not significant. The prevalence of circulating lipids, lipoproteins and apolipoproteins was not statistically different between patients with irAEs and those without irAEs. Patients who had suffered from irAEs tended to have higher apoA1/(apoA1+apoB) ratio (0.58 vs 0.54, p.val = 0.040). However, the apoA1 ratio was not an ideal biomarker for predicting irAEs for the diagnosis of the univariate and multivariate models containing apoA1 ratio was unsatisfied.

3.7 High expression of APOB potentially predicted poor ICIs response in the validation cohorts

Expression of APOB which encodes apoB proteins was tested for validation in GSE126044, and GSE135222 cohorts. 16 advanced NSCLC patients including 7 LUAD and 9 LUSC treated by Nivolumab

were included in GSE126044. Expression of APOB tended to be higher in the NDB group in the LUAD group despite without statistical difference (Wilcox test, p.val = 0.191, **Figure S6A**). The 16 patients were divided into two groups according to the expression of APOB. LUAD patients with high expressed APOB tended to have shorter PFS (Log-rank test, 2.22 months vs 3.5 months, p.val = 0.018, **Figure S6B**). High APOB in LUAD tended to predict a short PFS of ICIs (HR = 2.86, 95%CI 0.82 - 10.02, p.val = 0.100). The tendency did not exist in the total cohort and the LUSC cohort (The total group: HR = 1.05, 95%CI 0.76 - 1.46, p.val = 0.77). 27 advanced NSCLC patients treated with anti-PD-1/PD-L1 were included in GSE135222. Expression of APOB was more likely higher in the NDB group (Wilcox-test, p.val = 0.356, **Figure S6C**). The low APOB group tended to have a longer PFS in the NSCLC patients without a statistical difference (Log-rank test, 2.95 months vs 1.47 months, p.val = 0.239, **Figure S6D**). Patients with higher APOB potentially indicated shorter duration of response to ICIs (HR = 1.08, 95%CI 0.99 - 1.18, p.val = 0.073).

3.8 High expression of APOB corresponded to suppressed TME in LUAD

391 LUAD patients without EGFR sensitive mutations and 494 LUSC patients were respectively classified into three clusters in the TCGA cohorts (**Figure S7A-D**). CYT represented the immune response of the tumor, which decreased from C2 to C3 (C2>C1>C3, **Figure S7 B, D**). C3 cluster was more likely to be an immunologically cold or non-inflamed tumor and was excluded since it potentially belongs to ICIs resistant subset. C2 cluster presented as an immunologically hot phenotype and was more likely to benefit from immunotherapies. C1 cluster couldn't be classified in C2 or C3 cluster for the high immune heterogeneity which tended to be the most frequent immunophenotype in routine clinic conditions.

277 patients with LUAD samples and 479 LUSC patients in C1 and C2 clusters were divided into $APOB^{high}$ and $APOB^{low}$ groups according to the expression of APOB. According to the classification, $APOB^{low}$ expressed more major histocompatibility complex class I (MHC-I) molecules (**Figure 7A**). The infiltration of T helper 2 cells (Th2) cells and effect memory CD4+ T cells (emCD4) and NK cells were also higher in the $APOB^{high}$ group (**Figure 7A**). TME estimated by MCPcounter showed that the fibroblasts and monocytic lineage cells were highly infiltrated in $APOB^{high}$ group, which is more likely to lead to immunosuppression by forming a barrier around the tumor cells (**Figure 7B**). Likewise, according to the cell component derived from the methylation data, despite the total immune cells showing no statistical difference, the fibroblasts were higher in the $APOB^{high}$ group (**Figure 7C**).

When it comes to the LUSC cohort, $APOB^{high}$ group tended to have higher infiltrated immune cells which potentially explain the reason why the difference of ICIs response in LUSC according to serum apoB containing proteins level was not consistent with LUAD (**Figure S8**).

Functional enrichment analysis between the $APOB^{high}$ and $APOB^{low}$ groups in the TCGA-LUAD cohort showed that apart from metabolic syndrome-associated pathways, many types of cancer-related

pathways were upregulated in $APOB^{high}$ group. TGF beta signaling pathway, Wnt signaling pathway, and adipocytokine signaling pathway were upregulated in $APOB^{high}$ patients, which indicated the potential crosstalk of metabolism and cancer (**Figure 8A**). Differential gene analysis demonstrated that the apoB containing lipoproteins receptors including low-density lipoprotein receptor-related protein 5 (LPR5) and LPR6 which involve in the endocytosis of apoB containing lipoproteins and mediate the downstream Wnt signaling were upregulated in the non-inflamed TME and NDB patients. LDLR gene was not highly expressed in the NDB patients and patients with non-inflamed TME (**Figure 8B-C**).

4 Discussion

This was the first study to evaluate the apoB containing lipoproteins and the efficacy of the combination regimens of ICIs and chemotherapy in NSCLC patients. In this study, we investigated the association between BMI, serum lipids, serum lipoproteins levels and the efficacy of ICIs in patients with NSCLC who received the combination of ICIs and chemotherapy for the first time regardless of the PD-L1 status. We demonstrated that BMI $\geq$ 25 kg/m^2 was a risk factor for the poor ICIs response and shorter PFS. Moreover, the elevated apoB containing lipoproteins including LDL-C and RC independently predicted the poor response to ICIs especially in BMI < 25 kg/m^2 and LUAD subgroup. Public data including GSE126044 and GSE135222 verified that patients with high APOB expression tended to have a worse ICIs response. The bioinformatic validation in TCGA cohorts suggested that patients with high expression of APOB manifested as suppressed TME in LUAD which potentially explained the reason of high levels of apoB containing lipoproteins led to poor ICIs response. Our study suggested the crosstalk between cholesterol metabolism and ICIs response and indicated the potential utility of the noninvasively measured biomarkers including LDL-C and RC for predicting the efficacy of ICIs and chemotherapy regimens.

With the lifestyle changes, the issues of excess weight and dyslipidemia worldwide are prominent. High BMI has been linked to a proinflammatory state, leading to a high risk of cancer, which lends further credence to the view that obesity may exert an immune-modulatory rather than a simply prognostic role (6, 7). A prior study suggested the positive effect of BMI on the treatment outcome in patients with advanced NSCLC treated with ICIs monotherapy. However, the results were not reproduced in patients treated with the combination of ICIs and chemotherapy (8).

In this study, we obtained the opposite results: high BMI is a risk indicator for the poor ICIs response in NSCLC patients who are treated with the combination regimen. Practically, the study about BMI and ICIs monotherapy conducted by Cortellini et al demonstrated that obesity was a positive indicator for the longer PFS and better objective response rate (ORR) in the ICIs cohort, while it was a conversely negative indicator in the chemotherapy cohort (6). The polarized results suggested that the obesity-associated immune regulation was radically distinct in the chemotherapy and immunotherapy, which might explain the converse result about the association of BMI and ICIs response obtained in the study. Additionally, the prevalence of the obesity in Asia is lower than that in American and European countries, only 5 patients

with BMI ≥ 30 kg/m^2 were included in the study, most patients were overweight and normal weight, and a similar condition existed in another Japanese researcher (6-8). In most studies, the overweight group showed no significant difference compared with the normal weight. Another Japanese researcher chose BMI ≥ 21.4 kg/m^2 as the cut point and distinguished this cohort as a potential ICIs monotherapy benefited subgroup, which indicated a race-fitted BMI threshold might be necessary ([34]). Despite the prevalence of BMI was not significantly different according to the irAEs in this study, past studies demonstrated that higher pre-treatment BMI elevated the occurrence of any grade of irAEs (9-10). Summarily, different from ICIs monotherapy, the overweight subgroup might not gain the benefit from the chemotherapy combining regimen in the Chinese NSCLC patients, whereas patients with a normal weight would be the lucky one.

Cholesterol is essential for maintaining the integrity and fluidity of membrane and other lipid microdomains named lipid rafts and is the precursor to support the synthesis of vitamin D, bile acid, and steroid hormones ([35]). ApoB containing lipoproteins including LDL-C and remnant lipoproteins, such as small VLDL, IDL, and chylomicron remnants are critical carriers of cholesterol ([36]). Unlike LDL-C, HDL-C is an apoA1 containing lipoproteins and acts as a cholesterol transporter to the major steroidogenic organs (17). It has been acknowledged that elevated apoB containing lipoproteins and decreased HDL-C are prevalent risk factors for atherosclerosis diseases (17).

Preclinical and clinical studies have shown that tumorigenesis and cancer progression occur accompanied by elevated cholesterol requirements manifesting as increased de novo biosynthesis, hydrolysis of cholesterol ester stores, and uptake from the circulation via LDL receptor (LDLR) mediated endocytosis. High cholesterol level was seen in the non-inflamed TME which manifested as CD8 +T cell exhaustion and upregulation of PD-1 expression on CD8 +T cells ([37]). Increased LDL-C and decreased HDL-C have been reported to be associated with an increased risk of cancer development and poor prognoses in many types of cancer (17, [38]). Recent studies demonstrated that the high level of pre-treatment serum cholesterol, LDL-C, and HDL-C indicated better ICIs response and longer PFS in the NSCLC patients treated with ICIs monotherapy, whereas showed no association with the efficacy of cytotoxic chemotherapy ([39]). However, the results remained controversial for the counteractive effects on anticancer immunity of HDL-C and LDL-C. Additionally, the association between serum cholesterol and the cholesterol-riched lipoproteins and ICIs treatment response in ICIs and chemotherapy combination is far from clear.

In our study, we demonstrated that the high cholesterol-riched apoB containing lipoproteins including LDL-C and RC could be promise indicators for poor ICIs response. Serum RC was superior in predicting the ICIs response (DCB/NDB) and the best response (active/non-active) with the satisfied discriminative ability and the well-fitting calibration curves, whereas serum LDL-C tended to work well in predicting the PFS of ICIs with the favorable diagnosis of DCA. Despite the groups being demographically balanced, the above analyses were all based on the multivariate regression with the confounders including gender, age, diabetes status and smoking history, and BMI category adjusted as dyslipidemia always occurs in

patients with obesity and diabetes mellitus. In the subgroup analysis, we found that the predictive ability of apoB containing lipoproteins was more obvious in the LUAD cohort and BMI normal cohort.

We further validated our results in two external cohorts including GSE126044, and GSE135222. As the apoB was the main component of apoB containing lipoproteins, the expression of APOB gene which encodes apoB protein was used as the substitution of serum apoB containing lipoproteins. Despite the sample size of the two cohorts being both small, the tendency of negative effect on ICIs response to apoB expression was prominent. GSE126044 provided the histology details of the 16 samples, whereas the influence of APOB expression on ICIs response seems more obvious in the 7 LUAD samples.

To explore the mechanism of the negative apoB containing lipoproteins on ICIs response, we further performed the bioinformatic exploration in the TCGA cohort. As LUAD patients with EGFR mutations had been widely acknowledged as the ICIs resistant subgroup, we exclude these patients. The measurement of TME of the LUAD and LUSC cohorts was based on both expression and methylation data. Unlike LUAD, LUSC is characterized by a high overall mutation rate and marked genomic complexity, which tended to inflame tumors. In our study, 277/391 LUAD samples and 479/494 LUSC samples were not immunologically cold tumors, which confirmed the previous viewpoint ([40]). The non-inflamed samples were excluded for their potential resistance to ICIs. The APOB high group had more fibroblasts infiltrated which potentially form the acquired stromal barrier to protect the effector cytotoxic immune cells from killing tumor cells. The immunosuppressive cells including Th2 cells and monocytic lineage cells and the decreased MHC I molecules of APOB high tumors might further lead to the immunologically cold TME which might be the potential mechanism of the group showing poor response to ICIs.

The further functional enrichment analysis in the TCGA-LUAD cohort showed that TGF-β signaling and Wnt signaling were upregulated in the APOB high group apart from participating in cancers and metabolism syndrome signalings. TME plays dynamic and sophisticated roles in facilitating tumor growth and drug responses. The elevated cholesterol metabolism reprogrammed immunosuppressive cells such as tumor-associated macrophages and fibroblasts. The cholesterol contained in apoB lipoproteins is delivered into cancer cells by binding to the LDL receptor (LDLR) family. Low-density lipoprotein-related protein 6 (LRP6) and its close homolog LRP5 belong to the LDLR family and are known as important regulatory factors of serum cholesterol by acting as the co-receptors of Wnt pathway ([41], [42]). This crosstalk provides the potential possibility to form the non-inflamed TME by high LDL-C cholesterol since the Wnt pathway is the vital upstream of TGF-β signaling which promotes the transformation of fibroblasts and suppresses immune responses.

The mevalonate pathway is a well-known process in cholesterol biosynthesis. 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is reduced by its reductase called HMG-CoA (HMGCR) to form mevalonate. HMGCR is the primary rate-limiting enzyme in cholesterol biosynthesis. Statins mainly target HMGCR to exert a cholesterol-lowering effect and decrease the serum apoB containing lipoproteins level to inhibit the cancer cells' cholesterol acquirement. Past studies suggested that the concomitant use of statins during ICI treatment showed a positive impact on treatment outcomes. Lipophilic statins enable better antigen

presentation and tumor-suppressive responses by involving in endosomal trafficking. More recent studies demonstrated that statins could inhibit the recruitment of pro-tumorigenic macrophages by suppression of CC chemokine receptor 1 (CCR1) ligand secretion. However, the statins exposure could elevate the risk of ICIs-associated myositis by evoking the anti-HMGCR antibodies ([43]). The experimental findings proposed that the prolonged atorvastatin treatment led to drug resistance and progression of lung adenomas into the invasive disease by the acquisition of additional stromal changes and non-inflamed TME. Therefore, despite statins in combination with chemotherapy and ICs regimens can be a promised anti-tumor strategy, if the combination will be used routinely remains controversial.

Our study acknowledges several limitations beyond the single-center study and the consequent central effect bias such as the baseline characteristics and the concomitant anti-cancer medication habits. The sample size in the one center is small for which the effect of apoB containing lipoproteins on many potentially meaningful subgroups was not observed. In our cohort, the BMI of all patients was more than 18.5 kg/m^2, and the apoB containing lipoproteins effect on the underweight patients could not be evaluated. In addition, as a likely result of the clinicians' attitude to reserve chemotherapy for fitter patients, the ECOG-PS of patients in our cohort were all within 0 to 1. Moreover, only 5 patients were obese and therefore were combined with the overweight group. More evaluations on the underweight and the obese groups were needed.

Secondly, apart from the selection bias such as the recall bias and investigation bias which could be attenuated by choosing the objective indicators and medical records, the retrospective design also caused the event effect bias. As the ICIs response could be obtained at least the 6-month observation and some patents stopped ICIs for irAEs or other reasons, we added the best response to ICIs as the supplement endpoint. Patients experiencing long duration of ICIs treatment and showing active response to ICIs were more likely to suffer from the recorded irAEs. Patients without irAEs might have two conditions: on the one hand, patients did not occur the irAEs, on the other hand, the patients did not reach the occurrence time of irAEs.

Thirdly, the concomitant medications of statins and metformin were not brought into research despite their roles in controlling dyslipidemia, as the main points of the study were to observe the effects of apoB containing lipoproteins and other serum lipids and lipoproteins on ICIs response no matter how to control and maintain. Additionally, the APOB expression was used as the substitution of apoB containing lipoproteins in the validation cohorts as the serum lipids and lipoproteins data were unavailable and apoB was the major apolipoproteins in the transport of circulating cholesterol. We speculated that the high cholesterol-riched apoB containing lipoproteins mediated the poor ICIs response by promoting the enrichment of fibroblasts and other immunosuppressive cells through LRP6/5-Wnt-TGF-β axis. The potential mechanism required more laboratory experiments to verify.

5 Conclusion

In the NSCLC population treated with the combination of chemotherapy and ICIs regimen, BMI ≥ 25 kg/m2, the elevated cholesterol-riched apoB containing lipoproteins including LDL-C and RC are independent risk indicators for the poor ICIs response. The elevated apoB containing lipoproteins might promote the infiltration of fibroblasts and other immunosuppressive cells through LRP6/5-Wnt-TGF-β axis to acquire resistance to ICIs. BMI and apoB containing lipoproteins are promise biomarkers in predicting the response to the chemotherapy plus ICIs regimen, however further large-scale validations are needed. The mechanisms beyond the apoB containing lipoproteins regulating the ICIs response need experimental exploration.

Abbreviation

NSCLC: Non-small cell lung cancer; LUAD: lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; ICIs: Immune checkpoint inhibitors; TME: Tumor microenvironment; PD-L1: Programmed death-ligand 1; CYT: Cytolytic activity; BMI: body mass index; CHO: cholesterol; TG: ApoB: apolipoproteins B; LDL-C: low-density lipoproteins cholesterol; HDL-C: High-density lipoprotein cholesterol; RC: Remnant cholesterol; OS: Overall survival time; PFS: Progression-free survival; DCB: Durable clinical benefit; NDB: Non-durable benefit; PR: Partial response; SD: Stable disease; PD: Progressive disease; DCA: Decision curve analyses; HR: Hazard ratios; OR: Odds ratio; ROC: Receiver operating characteristic; AUC: The area under the ROC curve; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus; GSEA: Gene set enrichment analysis; DEGs: Differentially expressed genes;

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

ZX-H and HJ-C contributed to the idea and design. YM-Z, YX-Y, XY-L and J-L contributed to the data acquisition. JB-Z, HJ-C evaluated the radiographic data independently. ZX-H, JQ-L, Y-W, J-L contributed to the statistical analyses. ZX-H, JB-Z and HJ-C participated in the manuscript drafting and revision. All authors contributed to the publication according to the ICMJE guidelines for the authorship. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethical approval statement

The study was carried out with the ethical approval of the institutional Ethics Committee of the Faculty of Medicine at China-Japan Friendship Hospital approval (2021-122-K80). Patient consent of ICIs and chemotherapy combination treatment were

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Tables

Table 1
Baseline clinical characteristics of the 99 NSCLC patients administrated by the combination of chemotherapy and ICIs regimens.

Variable	*Mean (SD)/Median (95% CI)/Frequency*	*Num*	*Variable*	*Mean (SD)/Median (95% CI)/Frequency*	*Num*
Age	64.0 (58.0–69.0)	99	**ICIs**		99
Gender		99	**Anti PD1**	65 (65.7%)	
Female	15 (15.2%)		**Anti PDL1**	31 (31.3%)	
Male	84 (84.8%)		**Multi**	3 (3.03%)	
Stage		99	**Line**		99
I - II	3 (3.03%)		**1**	68 (68.7%)	
III	24 (24.2%)		**2+**	27 (27.3%)	
IV	72 (72.7%)		**PreOC**	4 (4.04%)	
Smoking History:		97	**Best response**		89
N	24 (24.7%)		**Active**	62 (69.7%)	
Y	73 (75.3%)		**Non active**	27 (30.3%)	
DM		97	**Responsiveness**		75
N	79 (81.4%)		**DCB**	44 (58.7%)	
Y	18 (18.6%)		**NDB**	31 (41.3%)	
Histology		99	**AE**		99
LUAD	55 (55.6%)		**N**	61 (61.6%)	
LUSC	37 (37.4%)		**Y**	38 (38.4%)	
NSCLC	6 (6.06%)		**Serum cholestrol (CHO)**	4.57 (1.25)	93
NSCLC + SCLC	1 (1.01%)		**Total triglyceride (TG)**	1.59 (0.84)	93
BMI		99	**Low density lipoprotein cholesterol (LDL-C)**	2.93 (0.87)	93
normal	58 (58.6%)		**High density lipoprotein cholesterol (HDL-C)**	1.09 (0.27)	93
overweight	36 (36.4%)		**Remnant cholesterol (RC)**	0.54 (0.29)	93

Variable	*Mean (SD)/Median (95% CI)/Frequency*	*Num*	*Variable*	*Mean (SD)/Median (95% CI)/Frequency*	*Num*
obesity	5 (5.05%)		**Apolipoprotein B (APOB) (ApoB)**	0.96 (0.28)	73
KRAS	18 (100%)	18	**ApoA1**	1.16 (0.26)	73
TPS_22C3:		27	**ApoE**	3.50 (1.33)	65
< 1%	4 (14.8%)		**ApoC3**	8.62 (4.51)	50
1% – 49%	13 (48.1%)		**ApoC2**	3.82 (2.19)	50
≥ 50%	10 (37.0%)				

Table 2
Models containing remnant cholesterol (RC) to predict ICIs response (DCB/NDB) and the best response to ICIs (active/non-active). Model 1: the multivariate logistic model containing RC as the continuous form used to predict the ICIs response and the best response. Model 2: the multivariate logistic model containing RC as the category form used to predict the ICIs response and the best response. Model 3: the univariate logistic model containing RC as the continuous form used to predict the ICIs response and the best response. Model 4: the univariate logistic model containing RC as the category form used to predict the ICIs response and the best response.

Variable	*ICIs response*			*The best response to ICIs*		
	OR	**95%CI**	**P.val**	**OR**	**95%CI**	**P.val**
Model1						
RC	10.46	1.36–103.47	0.031	5.25	0.79–41.31	0.100
BMI ≥ 25 kg/m2	6.61	2.08–23.92	0.002	5.28	1.86–16.35	0.002
Model2						
Low RC	0.12	0.02–0.63	0.017	0.22	0.05–0.96	0.047
BMI ≥ 25 kg/m2	6.91	2.12–25.85	0.002	5.31	1.85–16.76	0.003
Model 3						
RC	7.34	1.25–53.15	0.035	4.69	0.90–26.88	0.071
Model 4						
Low RC	0.19	0.04–0.73	0.022	0.29	0.08–0.87	0.043

Figures

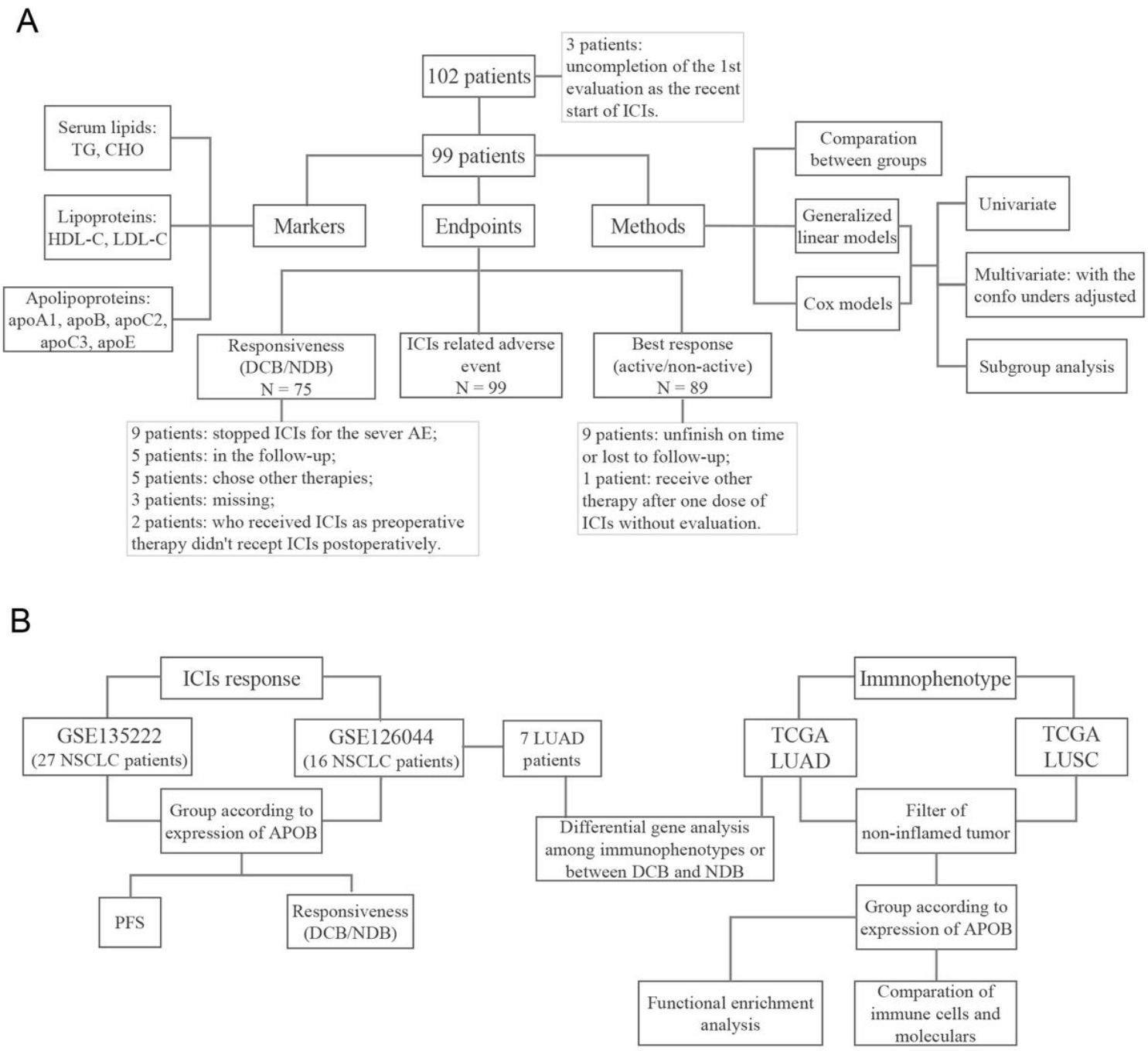

Figure 1

Workflow of the study. **(A)** Patient flow diagram. **(B)** Workflow of validation and mechanism exploration using public data.

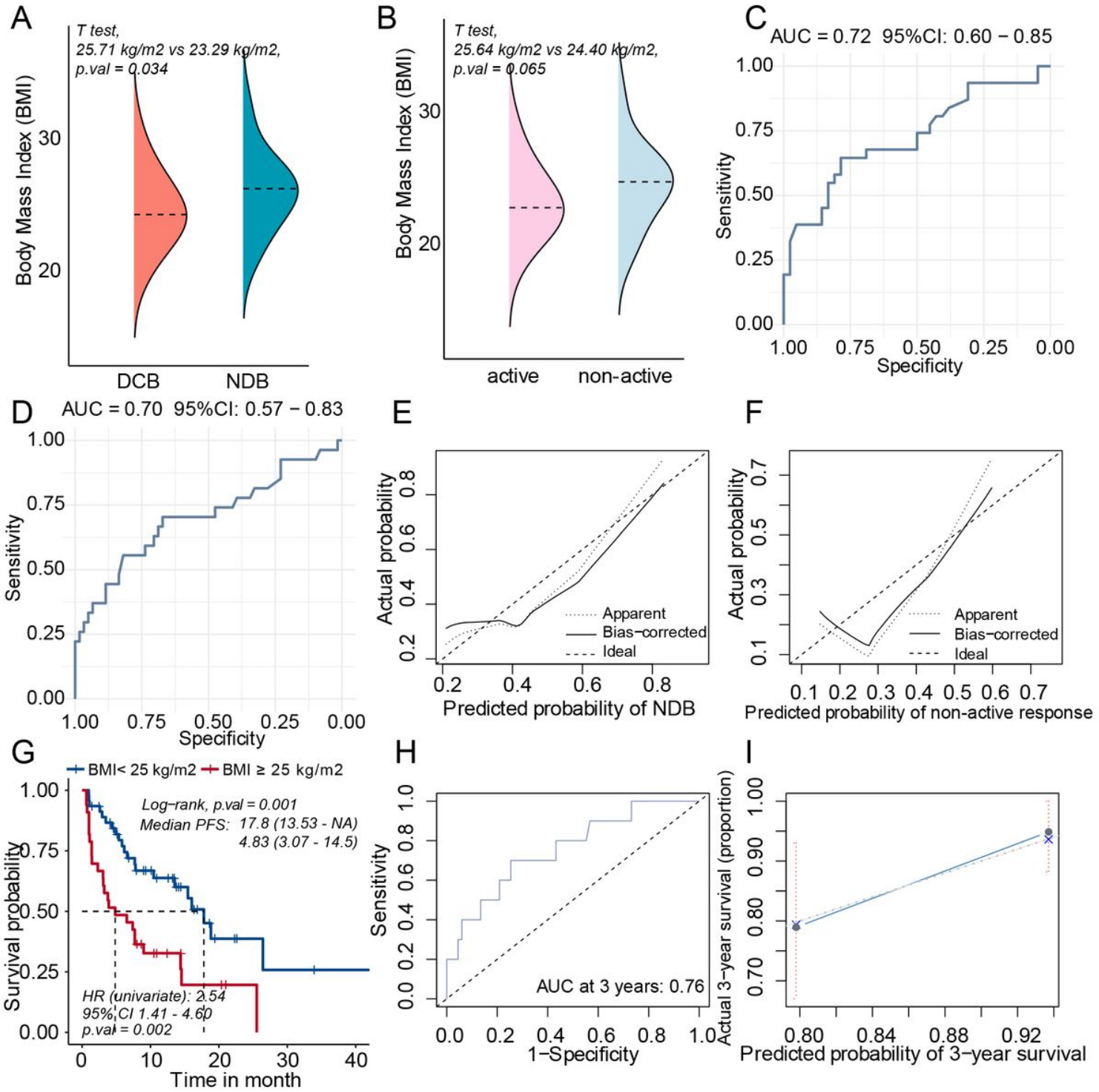

Figure 2

BMI ≥ 25 kg/m^2 predicted poor ICIs response in NSCLC patients. **(A)** Comparison of the BMI prevalence between patients with durable clinical benefit (DCB) and non-durable benefit (NDB). **(B)** Comparison of the BMI prevalence according to the best response to ICIs. **(C)** The receiver operating characteristic (ROC) curve of the multivariate model containing BMI ≥ 25kg/m^2 used to predict the ICIs response (DCB/NDB). (D) The ROC curve of the multivariate model containing BMI ≥ 25kg/m^2 used to predict the best response to ICIs response (active/non-active). **(E)** The calibration curve to diagnose the model containing BMI ≥

25kg/m^2 predict the ICIs response (DCB/NDB). **(F)** The calibration curve to diagnose the model containing BMI ≥ 25kg/m^2 predict the best ICIs response (active/non-active). **(G)** The Kaplan-Meier (KM) curve of BMI groups, NSCLC patients with BMI ≥ 25kg/m^2 treated with the chemotherapy and ICIs combination had shorter PFS compared with those with BMI< 25kg/m^2. **(H)** The time-dependent ROC curves of the multivariate Cox model containing BMI ≥ 25kg/m^2, the area under the ROC curve (AUC) at 3-year was 0.76. **(I)** The calibration curve of the multivariate Cox model containing BMI ≥ 25kg/m^2.

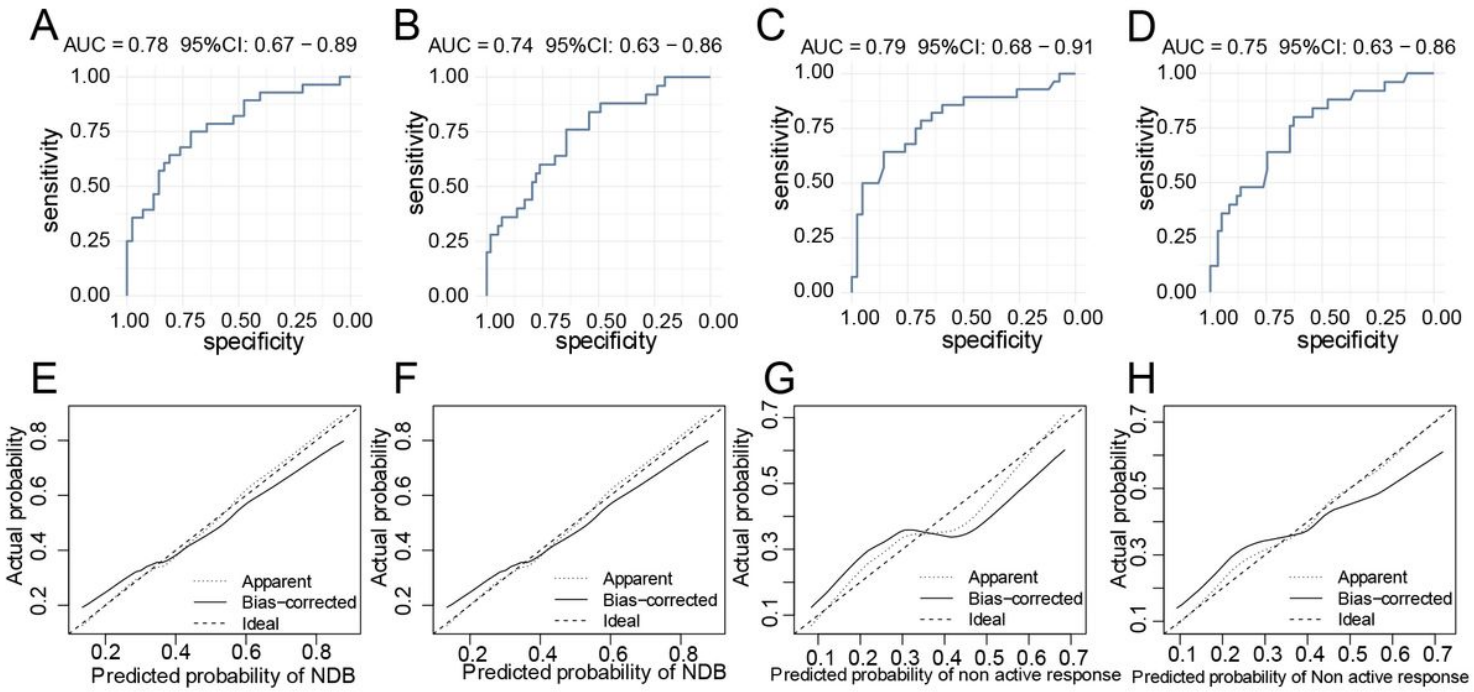

Figure 3

Diagnosis of multivariate models containing remnant cholesterol (RC) by the confounders including age, gender, smoking history, diabetes, and BMI adjusted. **(A)** The receiver operating characteristic (ROC) curve of the multivariate model containing RC as the continuous variate to predict the ICIs response (DCB/NDB). **(B)** The ROC curve of the multivariate model containing RC as the continuous variate to predict the best response to ICIs (active/non-active). **(C)** The ROC curve of the multivariate model containing low RC (category form) to predict the ICIs response. **(D)** The ROC curve of the multivariate model containing low RC (category form) to predict the best response to ICIs. **(E)** The calibration curve of the model containing RC in the continuous form to predict ICIs response. **(F)** The calibration curve of the model containing low RC to predict ICIs response. **(G)** The calibration curve of the model containing RC in the continuous form to predict the best response to ICIs. **(H)** The calibration curve of the model containing low RC to predict the best response to ICIs.

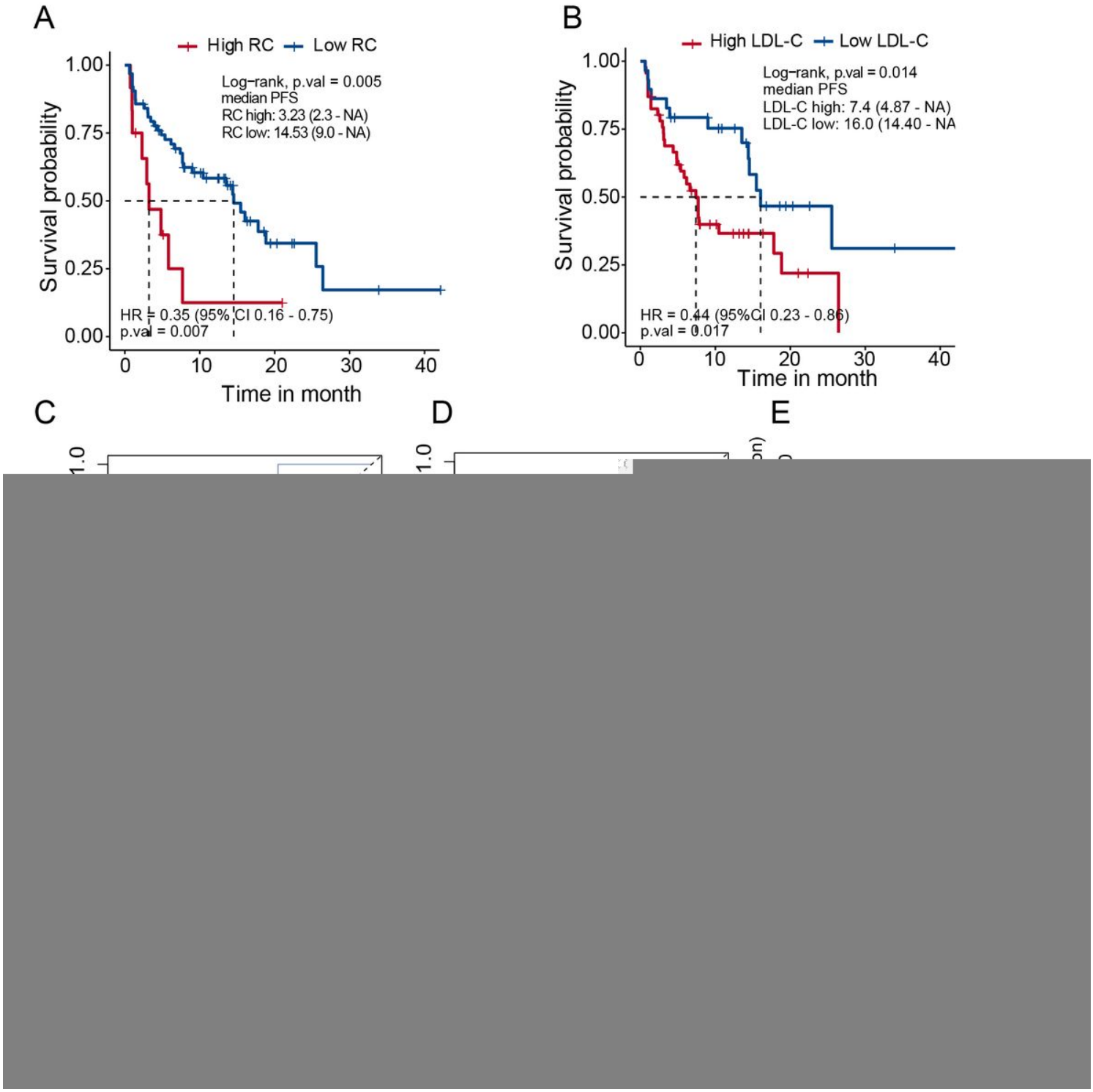

Figure 4

ApoB containing lipoproteins predict shorter PFS in the ICIs treated NSCLC patients. (A) The Kaplan-Meier (KM) curve of remnant cholesterol (RC) groups, ICIs treated NSCLC patients with high RC had shorter PFS compared with those with low RC. **(B)** The KM curve of low-density lipoproteins cholesterol (LDL-C) groups, ICIs treated NSCLC patients with high LDL-C had shorter PFS. **(C)** The receiver operating characteristic (ROC) curve of the multivariate Cox model containing LDL-C as the continuous variate to predict the PFS of ICIs treated NSCLC patients. **(D)** The ROC curve of the multivariate Cox model

containing low LDL-C (the category form) to predict the PFS of ICIs treated NSCLC patients. **(E)** The calibration curve of the multivariate Cox model containing LDL-C as the continuous variate at 3-year. **(F)** The calibration curve of the multivariate Cox model containing low LDL-C at 3-year. **(G)** The decision curve analysis (DCA) of the model comparison. Compared with the multivariate model which contained age, gender, smoking history, diabetes status, and BMI status (Model 1, the light blue line), the LDL-C-containing models added the extra predictive value to predict the PFS of ICIs. The model containing low LDL-C (the category form, Model 3, the dark pink line) acted superior to the model containing the LDL-C in the continuous form (Model 2, the dark blue line). The comparison among models was performed by ANOVA test.

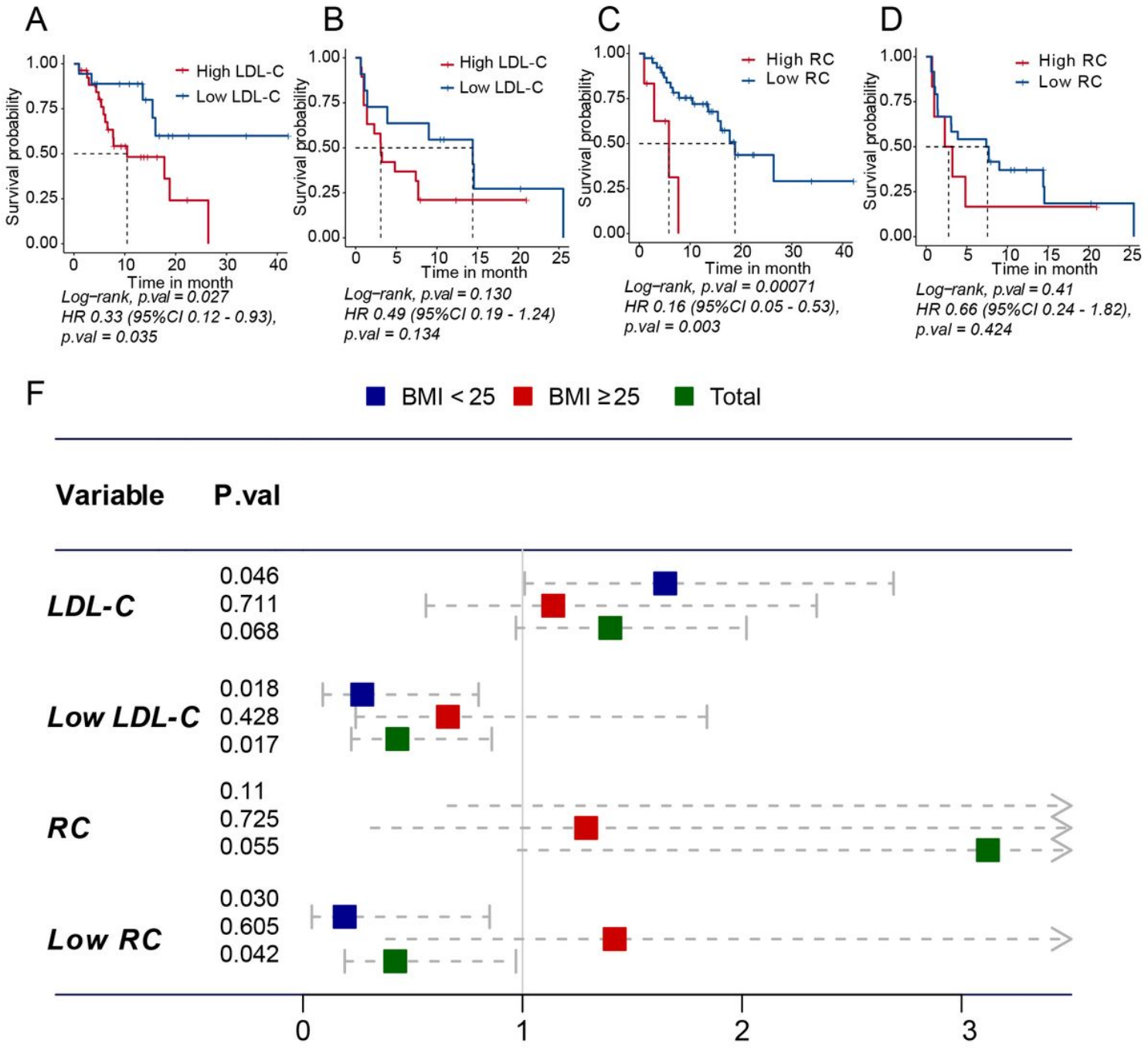

Figure 5

The prognostic value of cholesterol-riched apoB containing lipoproteins in the BMI subgroups. **(A)** The Kaplan-Meier (KM) curves showed low LDL-C predicted long PFS in the BMI < 25kg/m2 group (n = 58). **(B)** The KM analysis of the low LDL-C and the high LDL-C did not statistically distinct in the BMI ≥ 25kg/m2 group (n = 41). **(C)** The KM curves showed low RC predicted long PFS in the BMI < 25kg/m2 group. **(D)** The KM curve of RC groups did not statistically distinct in the BMI ≥ 25kg/m2 group. **(E)** Forest plot of the multivariate Cox models containing LDL-C or RC by the confounders (age, gender, smoking history, diabetes status) adjusted in the BMI groups.

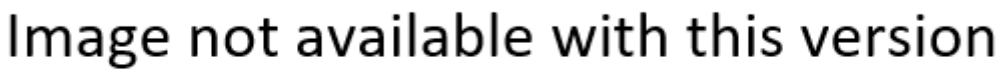

Figure 6

The prognostic value of apoB containing lipoproteins in the LUAD subgroup. **(A)** Comparison of serum cholesterol between the non-active group and the active group. **(B)** Comparison of serum low-density lipoproteins cholesterol (LDL-C) between the non-active group and the active group. **(C)** Comparison of serum remnant cholesterol (RC) between the non-active group and the active group. **(D)** Forest plot of the apoB containing lipoproteins (RC and LDL-C) predicting the duration of ICIs response in the LUAD cohort. The multivariate models were used to adjust the confounders including age, gender, smoking history, diabetes status, and BMI. **(E)** The Kaplan-Meier (KM) curves showed low LDL-C predicted longer PFS in the LUAD group (n = 55). **(F)** The KM analysis showed that the low RC predicted longer PFS in the LUAD group.

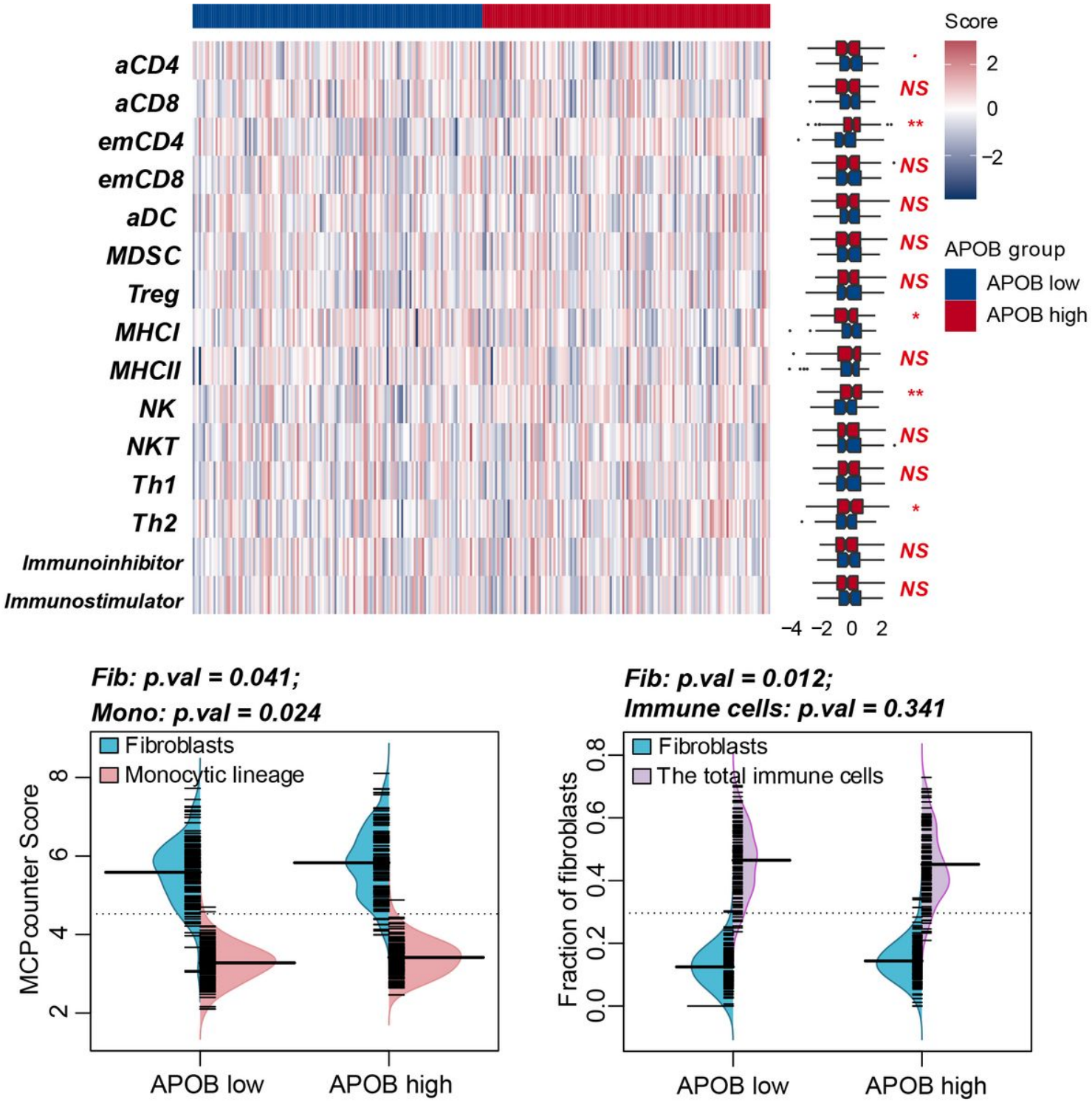

Figure 7

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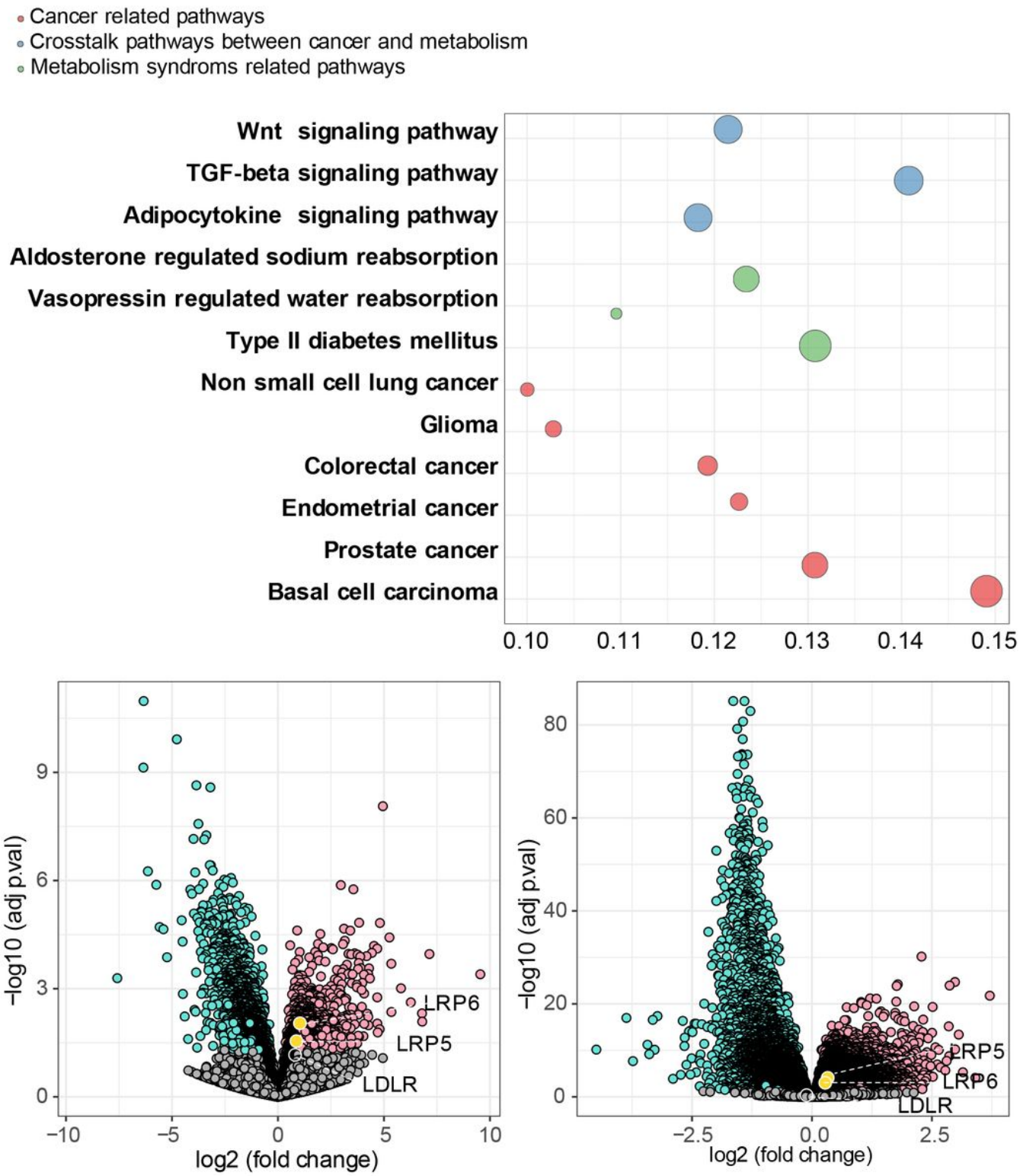

Figure 8

Tumor microenvironment (TME) analysis according to APOB expression in the TCGA-LUAD cohort. **(A)** Heatmap of TME measured by ssGSEA method. (B) Comparison of fibroblasts and monocytic lineage cells between APOB high and APOB low groups in TCGA-LUAD cohort. **(C)** Comparison of fibroblasts and total immune cells between $APOB^{high}$ and $APOB^{low}$ groups, the tumor infiltrated cells were remeasured by EpiDISH with methylation data.

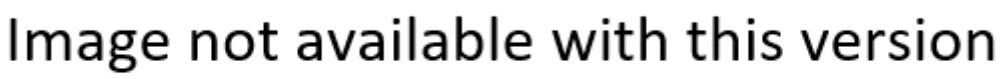

Figure 9

Functional enrichment and differential genes analysis according to the expression of APOB. **(A)** $APOB^{high}$ group tended to upregulate in the metabolic syndrome-associated pathways, many types of cancer-related pathways, TGF beta signaling pathway, Wnt signaling pathway, and adipocytokine signaling pathway. **(B)** NDB patients in the GSE126044 tended to have higher expression of low-density lipoprotein receptor-related protein 5 (LPR5) and LPR6 which belong to the family of apoB containing lipoproteins receptors and mediate the downstream Wnt signaling. **(C)** Compared with those with inflamed TME, patients with non-inflamed TME in the TCGA-LUAD cohort tended to have higher expression of LPR5 and LPR6.

Supplementary Files

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

- SupplementaryFiguresandTables.zip