Characteristics of genomic alterations and heavy metals in hypertensive patients with non-small cell lung cancer

XINBO ZHANG[1*], JIANHE YU[2*], HEPING SONG[1], YIMING WANG[1], MING WEN[3], LISHA JIANG[3] and HUIHUI JIANG[3]

[1]Department of Thoracic Surgery, First People's Hospital of Ping Ding Shan, Pingdingshan, Henan 467000, P.R. China; [2]Department of Oncology, Xinghua City People's Hospital, Xinghua, Jiangsu 225799, P.R. China; [3]Department of Medical Big Data Business, Zhangjiang Center for Translational Medicine, Shanghai Biotecan Pharmaceuticals Co., Ltd., Shanghai 200135, P.R. China

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Abstract. Both lung cancer and cardiovascular disease (CVD) are prevalent diseases that contribute to global mortality rates. Although individuals with CVD may face an elevated risk of cancer based on the presence of shared risk factors (such as tobacco smoking and excessive body weight), the roles of somatic mutations and heavy metal distributions remain unknown. The present study aimed to explore the differences in somatic mutations and heavy metal distributions between hypertensive patients and non-hypertensive patients in a cohort of patients with non-small cell lung cancer (NSCLC). Tumor tissue samples from 64 patients were analyzed using a next-generation sequencing panel consisting of 82 tumor-related genes through hybrid capture. Serum samples were also analyzed to determine the levels of 18 heavy metals using inductively-coupled plasma mass spectrometry. Among the 16 hypertensive patients, all patients (16/16; 100.00%) harbored 47 somatic mutations in 14 mutant genes, whereas 45 patients without hypertension (45/48; 93.75%) harbored 113 somatic mutations across 26 mutant genes (no mutations were detected in the remaining 3 patients). Among the 32 identified mutant genes in these two groups, FBXW7, CBR3, CDKN2A, HRAS, SMO and UGT1A1 were exclusively observed in patients with hypertension, while 18 mutant genes were only observed in patients without hypertension. No significant mutually exclusive interactions were found in hypertensive patients, but mutually exclusive interactions were observed between EGFR and STK11 (P=0.0240) and between STK11 and KRAS (P=0.0169) in non-hypertensive patients. 'Non-small cell lung cancer' was the top Kyoto Encyclopedia of Genes and Genomes pathway in hypertensive patients, whereas 'central carbon metabolism in cancer' was the top pathway in patients without hypertension. Moreover, the proportions of altered key signaling pathways and biological function categories shared between these two groups were 54.37% (56/103) and 21.62% (8/37), respectively. Furthermore, the levels of chromium (Cr) in the serum of hypertensive patients were notably elevated compared with those in patients without hypertension. In addition, significant negative correlations were observed between Cr and CEA, between CYFRA21-1 and Zn, and between NSE and As in hypertensive patients but not in non-hypertensive patients, indicating differing interactive profiles among the traditional serum biomarkers and heavy metals between these two patient groups. In summary, there were differences in genomic alterations, somatic interactions and the serum levels of Cr between patients with NSCLC with hypertension and patients with NSCLC without hypertension. Furthermore, patients with hypertension exhibited significant negative correlations between Cr and CEA, between CYFRA21-1 and Zn, and between NSE and As, suggesting that heavy metals may contribute to the occurrence of NSCLC with different hypertensive status.

Introduction

Lung cancer is an aggressive and globally prevalent disease, with ~2.48 million new cases and 1.82 million cancer-associated deaths worldwide in 2022 (1). Globally, it is the primary cause of cancer-associated death in men, whilst being the third most common type of cancer among women (behind breast and colorectal cancer) and the second most fatal behind breast cancer (2). Despite notable progress in the diagnostic and therapeutic methods of non-small cell lung cancer (NSCLC), the 5-year survival rates for lung cancer have only shown limited improvements over the past decades (3). For NSCLC specifically, survival rates improved from around 23% in the early 2010s to 26.4% in recent analyses (4). A considerable portion of patients with lung cancer are first diagnosed already at an advanced cancer stage, contributing to poor prognosis (3). Furthermore, although tobacco use persists as the

Correspondence to: Dr Huihui Jiang, Department of Medical Big Data Business, Zhangjiang Center for Translational Medicine, Shanghai Biotecan Pharmaceuticals Co., Ltd., 180 Zhangheng Road, Shanghai 200135, P.R. China
E-mail: hhjiang16@fudan.edu.cn

*Contributed equally

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primary risk factor, several other factors have been identified, including environmental exposures (such as biomass fuels, radon, industrial carcinogens and air pollution) and genetics (such as *EGFR* alterations) (5). Notably, ~25% patients with lung cancer are non-smokers (5,6). Consequently, exploring novel avenues to pinpoint high-risk cohorts presents itself as a promising strategy for the prevention and early detection of lung cancer to reduce both morbidity and mortality.

Cardiovascular disease (CVD) is a leading cause of global mortality, with an increase from 12.1 million in 1990, reaching 18.6 million in 2019 in 204 countries (7). In particular, hypertension is a key contributor of CVDs that has emerged as the primary driver of mortality, accounting for 7.6 million deaths annually worldwide reported by the World Health Organization in 2009 (8). The incidence of hypertension has increased two-fold from 1975 (594 million) to 2015 (1.13 billion) in 200 countries, largely due to an aging population (9). Individuals with CVD may face an elevated risk of cancer, given the presence of shared risk factors, such as tobacco smoking and excessive body weight, coupled with common pathogenic mechanisms, including inflammation, hypoxia and clonal hematopoiesis (10-14). Notably, individuals with CVD exhibit a 67% increased susceptibility to lung cancer and a 95% elevated risk of lung cancer-related mortality (15). Among male individuals who smoke and have hypertension, an association has been found between high blood pressure levels and an elevated risk of developing lung cancer (16). Moreover, findings from a Dutch study revealed that women with hypertensive disorders faced a 2.19-fold increased risk of mortality compared with those without such conditions in lung cancer (17). A close link between high blood pressure and lung cancer mortality was also observed in a South Korea study (18). However, these studies were epidemiological, not genetic. To the best of our knowledge, it remains to be determined whether and how high blood pressure can affect somatic mutations in patients with NSCLC.

Environmental exposures, including biomass fuels, arsenic (As), radon, industrial carcinogens and air pollution, can all contribute to the morbidity and mortality associated with lung cancer (5). Several studies have posited an association between environmental exposure to heavy metals [such as As, cadmium (Cd), chromium (Cr), copper (Cu), iron, mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn)] and the onset of lung cancer (19-23). Perturbations in metal homeostasis have the potential to induce oncogenic signaling pathways, impede DNA repair mechanisms, increase oxidative stress and alter epigenetic inheritance (19-22). Additionally, exposure to heavy metals, such as As, Cd, Hg and Pb, has been associated with elevated blood pressure levels (24-27). The underlying mechanisms involve oxidative stress, compromised nitric oxide signaling, altered vascular responses to neurotransmitters, disruptions in vascular muscle Ca^{2+} signaling, renal damage and interference with the renin-angiotensin system (24-26,28-31). However, previous epidemiological studies have primarily focused on the associations between heavy metals and the occurrence of a single disease (24-27), whilst, to the best of our knowledge, no studies have assessed the distributional differences in heavy metals between patients with NSCLC with and without hypertension.

Therefore, in the present study, targeted next-generation sequencing (NGS) of 82 tumor-associated genes and inductively-coupled plasma mass spectrometry (ICP-MS) detection of 18 heavy metals were performed on tissue DNA and serum samples, respectively, from patients with NSCLC with and without hypertension. The primary objectives of the present study were to elucidate the following: i) Disparities in genomic alterations, somatic interactions and Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enriched signaling pathways between patients with NSCLC with and without hypertension; ii) heavy metal profiles in patients with NSCLC with or without hypertension; and iii) how the different heavy metals associate with somatic mutations, demographics and clinical characteristics, in addition to other heavy metals in patients with NSCLC with hypertension.

Materials and methods

Patients and sample collection. A total of 64 patients with NSCLC from the Department of Thoracic Surgery at The First People's Hospital of Ping Ding Shan (Pingdingshan, China) were recruited between October 2019 and October 2021. The pathological diagnosis was confirmed by three separate pulmonary pathologists (XZ, HS and YW) using The Fourth Edition of the World Health Organization Classification of Lung Tumors (32). The NGS data were acquired from 64 formalin-fixed and paraffin-embedded (FFPE) tumor specimens. The tumor tissue specimens were fixed with 10% neutral buffered formalin at room temperature (~25˚C) for 24-48 h immediately after surgical removal. The present study adhered to the Code of Ethics established by the World Medical Association (Declaration of Helsinki) (33), and was approved by the Medical Ethics Committee of First People's Hospital of Ping Ding Shan (approval no. PYLL20190806; Pingdingshan, China).

The inclusion criteria were: i) Age >18 years; ii) an initial diagnosis of NSCLC by histological examination; iii) an enhanced CT scan of the chest and abdomen, MRI of the brain, and whole-body bone scan (emission CT) results were available; iv) diagnosed primary NSCLC; and v) no history of tumor treatment.

The exclusion criteria were: i) Patients had been subjected to tumor treatments, including chemotherapy, radiotherapy, targeted therapy or immunotherapy; ii) history of exposure to trace elements, toxic elements or heavy metals; iii) patients used antioxidants, vitamins or dietary supplements; iv) patients had undergone surgery within the past year; v) patients had comorbidities, such as autoimmune disease, gout, chronic liver disease, chronic kidney disease, protein-energy malnutrition, thyroid disease or vitamin A/D deficiency; or vi) patients had additional comorbidities deemed inappropriate for the present study by the research team, such as uncontrolled diabetes, psychiatric disorders, and substance abuse.

Assessment of hypertension. To be diagnosed with hypertension, patients must have met at least one of the following three criteria: i) Affirmative response from the patient to the question of whether they had a prior diagnosis of hypertension from a medical professional; ii) individuals exhibiting a

systolic blood pressure (SBP) of ≥140 mmHg and/or diastolic blood pressure (DBP) of ≥90 mmHg; and iii) individuals currently using blood pressure-lowering medications, including angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, diuretics and beta-blockers. Hypertensive participants were categorized into three hypertension grades based on their blood pressure readings: i) Grade 1, 140≤ SBP <160 mmHg and/or 90≤ DBP <100 mmHg; ii) grade 2, 160≤ SBP <180 mmHg and/or 100≤ DBP <110 mmHg; and iii) grade 3, SBP ≥180 mmHg and/or DBP ≥110 mmHg (34). In addition, the categorized criteria of patients with optimal, normal and high normal blood pressure readings were as follows: Optimal, SBP <120 mmHg and DBP <80 mmHg; normal, 120≤ SBP <130 mmHg and/or 80≤ DBP <85 mmHg; high normal, 130≤ SBP <140 mmHg and/or 85≤ DBP <90 mmHg.

DNA extraction and quality control. The extraction of genomic DNA (gDNA) from the FFPE tumor specimens was performed using a GeneRead DNA FFPE Kit (Qiagen GmbH). gDNA quantity and purity were assessed using a Qubit® 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). The Agilent 2100 Bioanalyzer instrument (Agilent Technologies, Inc.) was used to evaluate DNA integrity using High Sensitivity DNA Reagent (Agilent Technologies, Inc.).

Library preparation, hybridization capture and Illumina sequencing. Firstly, 300 ng gDNA from each FFPE tumor specimen was mechanically fragmented by an E220 focused ultrasonicator Covaris (Covaris, LLC) at 75W peak incident power, 20% duty factor, 1000 cycles per burst and a 7˚C water bath temperature for 95 sec treatment time. The DNA fragments were broken into fragments of 150-200 bp. Secondly, the KAPA library preparation kit (cat. No. KK8500; Kapa Biosystems; Roche Diagnostics) was employed to construct libraries using 10-100 ng gDNA fragments. Finally, the xGen Lockdown Probe pool (Integrated DNA Technologies, Inc.) was used to capture the NGS libraries, followed by amplification of the captured DNA fragments using 1x KAPA HiFi Hot Start Ready Mix (Kapa Biosystems; Roche Diagnostics). The sequences of the forward and reverse primers were 5'-AATGATACGGCG ACCACCGAGATCTACAC-3' (Illumina P5 primer) and 5'-CAAGCAGAAGACGGCATACGAGAT-3' (Illumina P7 primer). The thermal cycling protocol was as follows: Initial denaturation step at 98˚C for 45 sec, followed by 13 cycles of 15 sec at 98˚C, 30 sec at 60˚C and 30 sec at 72˚C. The final extension was conducted at 72˚C for 60 sec. The fragment length of library amplification products was analyzed by Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) and High Sensitivity DNA Kit (cat. No. 5067-4626; Agilent Technologies, Inc.). The library concentrations were determined by Qubit® 3.0 Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and the final library concentration for sequencing was 1.8 pM. Illumina NextSeq CN500 platform (Illumina Inc.) and NextSeq CN500 Mid Output v2 Kit (150 cycles) (cat. no. R0151; Illumina Inc.) were adopted to perform sequence with a 75 bp pair end mode.

Bioinformatics analysis. Fastp software (v.0.23.2; OpenGene Bioinformatics) was applied to remove low-quality reads [the proportion of low-quality bases (quality below Q15) in a read exceeds 40%], resulting in clean data. Burrows-Wheeler-Alignment Tool (BWA v.0.7.12; Wellcome Trust Sanger Institute) was used to align all filtered reads using the human genome as the reference (University of California Santa Cruz ID: hg19). Subsequently, Picard tools (v.1.130; Broad Institute) and Genome Analysis Toolkit (v.3.2.2; Broad Institute) were used to remove duplicates, perform indel realignment and recalibrate the base quality scores (35,36). The CollectTargetedPcrMetrics tool of Genome Analysis Toolkit was employed to generate quality statistics. Finally, VarDict (v.1.6.0; GitHub, Inc.) was used to verify single nucleotide variations and insertions/deletions (37).

ANNOVAR (v. 20210202; https://annovar.openbioinformatics.org/en/latest/) software was used to annotate somatic mutations (38). The identification of somatic mutation candidates was performed using the following filtering criteria: i) Removal of the variants coverage depth (VDP) <10; ii) variant sites with a mutant allele frequency (MAF) >0.001 in the 1,000 Genomes databases were removed (1,000 Genomes Project Consortium; https://www.internationalgenome.org/); iii) variant sites with a MAF ≥0.001 and <0.1 in the 1,000 Genomes databases, accompanied by COSMIC (http://cancer.sanger.ac.uk/cosmic) evidence, were retained; iv) variations in the exonic or splicing region (10 bp upstream and downstream of splicing sites) were retained; v) synonymous mutations were excluded; vi) variants with an unknown classification were excluded; and vii) functionally benign variant sites as predicted by PolyPhen 2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) were excluded. KEGG pathway and GO term enrichment analysis were performed using the R package clusterProfiler (v3.10.1), and P<0.05 was considered to indicate a statistically significant difference (39).

Detection of traditional serum biomarkers. To obtain serum, a minimum of 10 ml whole blood from each patient at initial diagnosis was centrifuged at 1,690 x g for 10 min at 4˚C. Serum samples were stored at -80˚C. The preparation of standard solutions of CEA, cytokeratin-19 fragments (CYFRA21-1) and neuron-specific enolase (NSE) was performed by serial dilution with a buffer solution (pH 7.5). Chemiluminescence immunoassays were used to measure the concentrations of these three aforementioned conventional serum biomarkers for tumors, as previously described (40,41). An automated chemiluminescence immunoassay analyzer (COBAS 8000 E 801; Roche Diagnostics GmbH) was used for analysis, with Carcinoembryonic Antigen (CEA) Assay Kit (Electrochemiluminescence Method) (cat. no. 07027079190), Cytokeratin 19 Fragment (CYFRA 21-1) Assay Kit (Electrochemiluminescence Method) (cat. no. 07299966190) and Neuron-Specific Enolase (NSE) Assay Kit (Electrochemiluminescence Method) (cat. no. 07299982190), all sourced from Roche Diagnostics GmbH.

Detection of heavy metals. The serum levels of 18 heavy metals [As, barium (Ba), Cd, cobalt (Co), Cr, Cu, gallium, Hg, manganese (Mn), Ni, Pb, antimony (Sb), selenium (Se), stannum (Sn), strontium (Sr), thallium (Tl), vanadium and

Zn] were determined by ICP-MS, utilizing an Agilent 7800 instrument (Agilent Technologies, Inc.) with an SPS 4 Series autosampler (Agilent Technologies, Inc.) and a peristaltic pump for injecting samples. The Agilent ICP-MS 7800 instrument (Agilent Technologies, Inc.) was chosen for its capability to analyze multiple elements, with specific detection procedures detailed in the manufacturer's instructions (42).

For sample preparation, ≥2 ml whole blood was collected from each patient at initial diagnosis and centrifuged at 1,690 x g for 10 min at room temperature to obtain the serum. Serum samples were stored at -20˚C until use. Subsequently, 500 μl serum and 500 μl internal standard solution (10 μg/ml; cat. no. 5191-4570; Agilent Technologies, Inc.) were added to 4,000 μl diluent consisting of 0.1% nitric acid (cat. no. 1.00456; MilliporeSigma) and 0.1% Triton™ X-100 (cat. no. 93443; MilliporeSigma). All components were then mixed and stored at 4˚C until analysis.

The analysis of 18 heavy metals was performed using helium gas mode, with the instrument parameters shown in Table I. Sample analysis was preceded by an autotuning system using a tuning solution (1 μg/l cerium, Co, lithium, magnesium, Tl and yttrium; cat. no. 5185-5959; Agilent Technologies, Inc.), with the following major parameters: i) The mass 7 sensitivity should be >3,000 counts/sec; ii) the mass 89 sensitivity should be >10,000 counts/sec; iii) the mass 205 sensitivity should be >6,000 counts/sec; iv) for the aforementioned measurements, the relative standard deviation should be <15%, the oxide ratio <2%, the doubly charged ratio <3% and the peak height at 10% width between 0.65 and 0.8; and v) in helium mode, the Co intensity count should be >2,400. System control and data acquisition and processing were conducted using the MassHunter 4.4 Workstation® version C.01.04 software (Agilent Technologies, Inc.).

Table I. Inductively coupled plasma mass spectrometry instrument parameters for helium gas mode.

A, Plasma parameters

Parameter	Value
RF power, W	1,550
RF matching, V	1.55
Sample depth, mm	8.0
Carrier gas, l/min	0.7
Option gas[a]	0
Nebulizer pump, rps	0.25
S/C temperature, ˚C	2
Gas switch	Makeup gas
Makeup/dilution gas, l/min	0.38

B, Lens parameters

Parameter	Value
Extract 1, V	0.0
Extract 2, V	-180.0
Omega bias, V	-110
Omega lens, V	10.5
Cell entrance, V	-50
Cell exit, V	-70
Deflect, V	-2.9
Plate bias, V	-50

C, Cell parameters

Parameter	Value
Use gas	True
Helium flow, ml/min	4.5
H_2 flow, ml/min	0.0
Third gas flow	0
OctP bias, V	-18.0
OctP RF, V	180
Energy discrimination, V	2.0

[a]The parameter of option gas indicates the argon-oxygen mixture used for organic samples. OctP, octopole; RF, radio frequency; S/C, sample/cone.

Statistical analysis. The 'maftools' package (version 2.6.05) within the R software environment (version 4.0.3; R Core Team; https://www.r-project.org) was used to visualize genomic landscapes, lollipop plots and spectra of co-occurring and mutually exclusive genomic alterations (43). Statistical differences in the categorical variables between patients with and without hypertension were assessed using χ^2 test or Fisher's exact test in SPSS (version 22.0; IBM Corp.). Continuous variables are presented as the median and interquartile range (IQR), where comparisons between two groups were conducted using an unpaired Student's t-test or a Mann-Whitney U test using GraphPad Prism (version 7.0; Dotmatics). Spearman's rank correlation analysis by the R software (version 4.0.3; R Core Team; https://www.r-project.org) was used to assess the correlations among age, BMI, three traditional serum biomarkers and 18 heavy metals. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. In the present study, the number of patients with primary NSCLC with stage 0, I, II, III and IV was 1, 33, 7, 17 and 6, respectively. Regarding hypertension status, 16 patients were confirmed to have hypertension (aged 39-82 years, median 64.50; referred to as Hyp group), and 48 patients did not have hypertension (aged 33-80 years, median 61.50; referred to as non-Hyp group). All 16 hypertensive patients were taking antihypertensive drugs, where the numbers of patients with different hypertension grades were 0 in optimal, 4 in normal, 3 in high normal, 5 in grade 1, 4 in grade 2 and 0 in grade 3. Among the 64 patients (Hyp group vs. non-Hyp group, 16 vs. 48), serum samples from 56 patients (Hyp group vs. non-Hyp group, 14 vs. 42) were used for the detection of conventional serum biomarkers, whereas serum samples from 62 patients (Hyp group vs. non-Hyp group, 16 vs. 46) were used for the assessment of 18 heavy metals. For

the unaccounted for serum samples, 2 were excluded due to hemolysis during storage, and 6 patients provided an insufficient volume of serum, making it impossible to perform all planned analyses. No significant differences were observed regarding age, height, weight, BMI, sex distribution, the incidence of EGFR or TP53 mutations, pulmonary infection history, smoking history, drinking history, cancer staging, blood vessel invasion, nerve invasion, distant metastasis status, CEA, CYFRA21-1 and NSE levels between the Hyp group and the non-Hyp group, but significant differences were observed for other chronic diseases (including coronary heart disease, diabetes, atrial fibrillation, Parkinson's disease and chronic gastritis) (P<0.001; Table II).

Disparities in genomic alterations between patients with NSCLC with and without hypertension. Despite the considerable advantages observed when targeted therapies (such as gefitinib, afatinib and osimertinib) are used for the treatment of patients with NSCLC harboring driver gene mutations (44,45), there remains a knowledge gap regarding the differences in genomic profiles between individuals with hypertension and those without. To explore these differences, somatic mutations in the tumor tissues of 16 hypertensive patients and 48 non-hypertensive patients were examined using an NGS panel comprising 82 tumor-related genes, which were related to the occurrence, progression and treatment of solid tumors, mainly derived from the NCCN clinical practice guidelines in oncology, the cancer knowledgebase database and the oncokb database (Table SI). In total, 47 somatic mutations involving 14 mutant genes were detected in all 16 patients with hypertension (100%). In comparison, 113 somatic mutations affecting 26 mutant genes were identified in 45 of the 48 patients without hypertension (93.75%; Fig. 1; Tables SII-IV). Among the identified mutant genes, *FBXW7*, *CBR3*, *CDKN2A*, *HRAS*, *SMO* and *UGT1A1* were exclusively observed in patients with hypertension, whilst 18 mutant genes (including KRAS, MAP2K1, RB1, CTNNB1, STK11, TPMT, NTRK1, CYP2D6, DPYD, KIT, PIK3CA, BRAF, ESR1, FGFR2, GNAS, NOS3, NOTCH1 and PDGFRA) were only observed in patients without hypertension.

In addition, although *EGFR*, *TP53*, *ERBB2*, *ALK*, *APC*, *FGFR3*, *RET* and *SOD2* mutations were detected in both groups, their mutation frequencies and sites markedly differed between the Hyp group and the non-Hyp group (Fig. S1; Table SV). For *TP53* mutations, whilst ~50% of the patients in both the Hyp group (56.25%; 9/16) and the non-Hyp group (50%; 24/48; Fig. 1), notable differences were identified in the mutation types and sites (Fig. S1). Specifically, except for the G245D mutation, the other 26 mutant sites differed between the two groups (Hyp group: R273L, A159P, T125K, E221Q, Q192X, D148H, C141W, P152T, 187_193del, and 187_192del; non-hyp group: S241F, R196G, D42fs, S241Y, L344P, E286G, R158L, C275F, 131_132del, T284P, Y236C, E298X, R196X, R306X, R273C, and V203M). Moreover, the mutation rate of *EGFR* was found to be higher in patients with hypertension (75.00%; 12/16) compared with those without (66.67%; 32/48).

Disparities in somatic interactions between patients with NSCLC with and without hypertension. In NSCLC, mutations in *EGFR* and *KRAS* tended to occur independently, with instances of patients harboring both mutations exhibiting resistance to EGFR-tyrosine kinase inhibitors (46). In the present cohort, the somatic interactions identified in hypertensive patients differed from those observed in non-hypertensive patients. In the non-Hyp group, mutually exclusive interactions were observed between *EGFR* and *STK11* (P=0.0240; Fig. 2B; Table SVI). However, no significant mutually exclusive interactions were found in the Hyp group (Fig. 2A; Table SVI). By contrast, notable disparities were observed in the sets of co-occurring genes between the two groups. *APC* and *ALK* (P=0.0588), *FGFR3* and *ALK* (P=0.0588), *HRAS* and *ALK* (P=0.0588), and another 13 pairs of genes were markedly co-occurring in hypertensive patients (Fig. 2A; Table SVI). The significant sets of co-occurring genes in patients without hypertension were as follows: *NTRK1* and *KIT* (P=0.0010), *KRAS* and *STK11* (P=0.0169), *RET* and *ALK* (P=0.0217) and another 22 pairs of genes (Fig. 2B; Table SVI).

Disparities in key signaling pathways and biological functional categories between patients with NSCLC with and without hypertension. To obtain a deeper understanding of the functional biological distinctions between the Hyp and the non-Hyp group, KEGG and GO analyses were performed. The top 10 KEGG signaling pathways based on both gene counts and P-values were identified, where the majority of the signaling pathways found were closely associated with cancer (Fig. 3A and B; Table SVII). 'Non-small cell lung cancer' was the top KEGG pathway in hypertensive patients, whereas 'central carbon metabolism in cancer' was the top pathway in patients without hypertension. In the GO enrichment analysis, the most prevalent functional category in both groups was 'transmembrane receptor protein tyrosine kinase activity' (Fig. 3C and D; Table SVII). In addition, the proportions of altered signaling pathways and altered functional terms shared between these two groups were 54.37% (56/103) and 21.62% (8/37), respectively (Fig. 3E). Unexpectedly, all functional terms in the Hyp group were also identified in the non-Hyp group.

Disparities in levels of heavy metals between patients with NSCLC with and without hypertension. To further examine the differences between the groups of patients with NSCLC based on hypertension status, a comparative analysis of three conventional serum biomarkers between individuals with hypertension (n=14) and those without hypertension (n=42) was performed. The serum levels of CEA, CYFRA21-1 and NSE were all found to be similar between the Hyp group and the non-Hyp group (Table SVIII and IX). Subsequently, the serum concentrations of 18 heavy metals in the Hyp group (n=16) and the non-Hyp group (n=46) were compared, revealing a significant difference in Cr, with no significant difference found in the other 17 heavy metals (Fig. 4). The median concentrations (IQR) for Cr were as follows: 2.635 μg/l (1.678-3.438 μg/l) in the Hyp group and 1.97 μg/l (1.525-2.483 μg/l) in the non-Hyp group (Fig. 4E).

Correlations among somatic mutations, demographic and clinical characteristics, traditional serum biomarkers and heavy metals in patients with NSCLC with hypertension. In the male group, the proportion of smokers (P=0.0049) and

Table II. Clinical characteristics of patients categorized based on hypertension status.

	Hypertension status			
Clinical characteristics	No. of patients (n=64)	Yes (n=16)	No (n=48)	P-value
Sex, N				0.561
Male	25	5	20	
Female	39	11	28	
EGFR mutations, N				0.757
Yes	44	12	32	
No	20	4	16	
TP53 mutations, N				0.776
Yes	33	9	24	
No	31	7	24	
Pulmonary infection history, N				0.667
Yes	8	1	7	
No	56	15	41	
Other chronic disease, N				<0.001[a]
Yes	7	7	0	
No	57	9	48	
Smoking history, N				0.093
Former	5	3	2	
Now	11	1	10	
Never	48	12	36	
Drinking history, N				0.459
Former	2	1	1	
Now	8	3	5	
Never	54	12	42	
Cancer staging, N				0.428
0	1	1	0	
I	33	8	25	
II	7	1	6	
III	17	5	12	
IV	6	1	5	
Vascular invasion, N				0.498
Yes	15	5	10	
No	49	11	38	
Nerve invasion, N				>0.999
Yes	12	3	9	
No	52	13	39	
Distant metastasis, N				>0.999
Yes	6	1	5	
No	58	15	43	
CEA at baseline, N				>0.999
Normal, 0-4.3 ng/ml	42	10	32	
Elevated, >4.3 ng/ml	18	4	14	
Unknown	4	2	2	
CYFRA21-1 at baseline, N				0.121
Normal, 0-3.3 ng/ml	34	11	23	
Elevated, >3.3 ng/ml	24	3	21	
Unknown	6	2	4	

Table II. Continued.

Clinical characteristics	Hypertension status			P-value
	No. of patients (n=64)	Yes (n=16)	No (n=48)	
NSE at baseline, N				0.431
Normal, 0-15.2 ng/ml	49	13	36	
Elevated, >15.2 ng/ml	9	1	8	
Unknown	6	2	4	
Median age, years (IQR)	62.000 (54.250-65.000)	64.500 (58.250-75.750)	61.500 (54.000-65.000)	0.056
Median height, m (IQR)	1.600 (1.600-1.680)	1.625 (1.600-1.698)	1.600 (1.600-1.675)	0.700
Median weight, kg (IQR)	60.500 (55.000-69.750)	63.500 (58.500-74.000)	60.000 (55.000-68.500)	0.206
Median BMI, kg/m^2 (IQR)	23.220 (21.340-25.800)	23.550 (21.900-25.790)	22.940 (20.600-25.800)	0.199

[a]P<0.05. CYFRA21-1, cytokeratin-19 fragments; IQR, interquartile range; NSE, neuron-specific enolase.

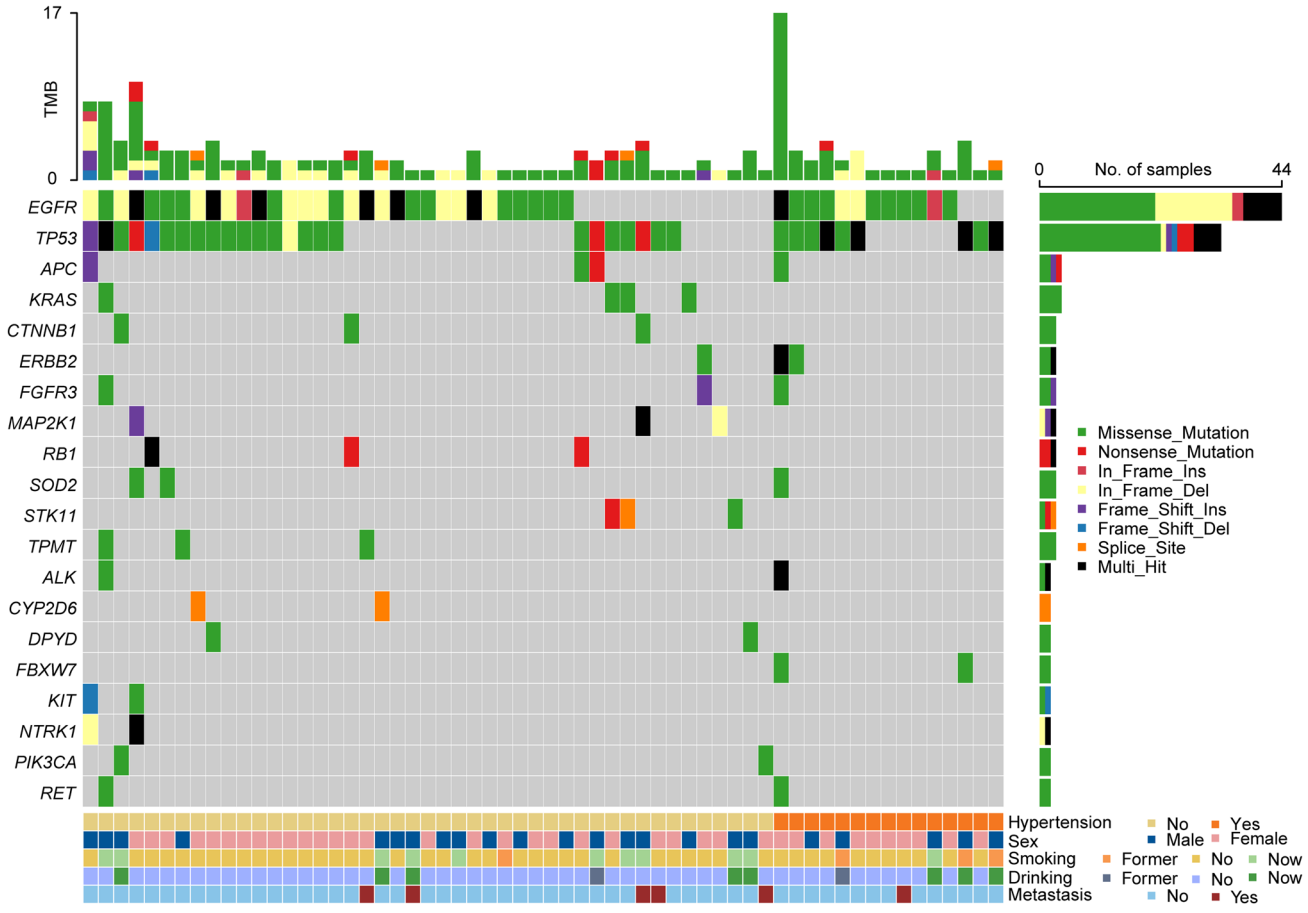

Figure 1. Somatic mutation landscape of tumor tissue DNA in a cohort of patients with non-small cell lung cancer (n=64). Patients were plotted along the x-axis (one square represents 1 patient), with mutant genes ordered based on their frequency. The right panel displayed the top 20 mutant genes. The upper panel illustrates the TMB, presented as mutations per megabase. Del, deletion; Ins, insertion; TMB, tumor mutation burden.

drinkers (P=0.0049) were significantly higher than those in the female group (Fig. S2; Table SVIII). Compared with the non-smoking group, the proportion of drinkers in the smoking group was significantly higher (P=0.0009; Fig. S2; Table SVIII). In addition, compared with that in patients without *EGFR* mutations, a significant decrease in Sn levels was observed in patients with *EGFR* mutations (P=0.0110) according to the two-tailed Mann-Whitney U test (Fig. S3; Table SVIII). However, none of the three traditional serum biomarkers, CEA, CYFRA21-1 and NSE, or 18 heavy metals exhibited significant differences between patients with and without *TP53* mutations, between patients with and without other chronic diseases, between male and female sex, between non-smokers and smokers or between non-drinkers and

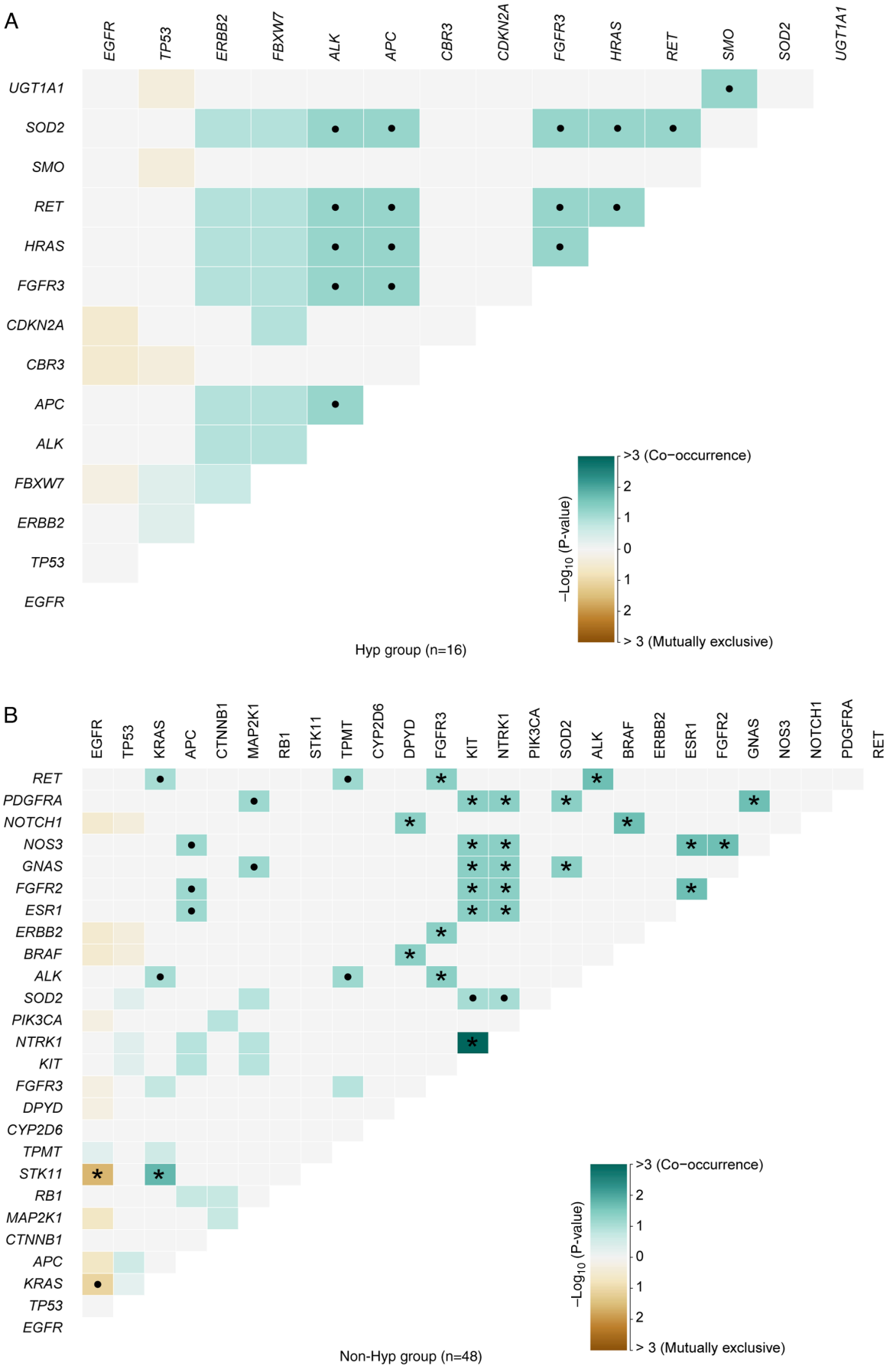

Figure 2. Spectra of co-occurring and mutually exclusive genomic alterations in patients with non-small cell lung cancer (A) with and (B) without hypertension. Green indicates co-occurrence and brown indicates mutually exclusive. In the non-Hyp group, the mutually exclusive interaction was only observed between *EGFR* and *STK11*, while there were 25 significant sets of co-occurring genes, including *NTRK1* and *KIT*, *KRAS* and *STK11*, *RET* and *ALK*, and another 22 pairs of genes. *P<0.05. •P<0.1. Hyp group, hypertension group; non-Hyp group, non-hypertension group.

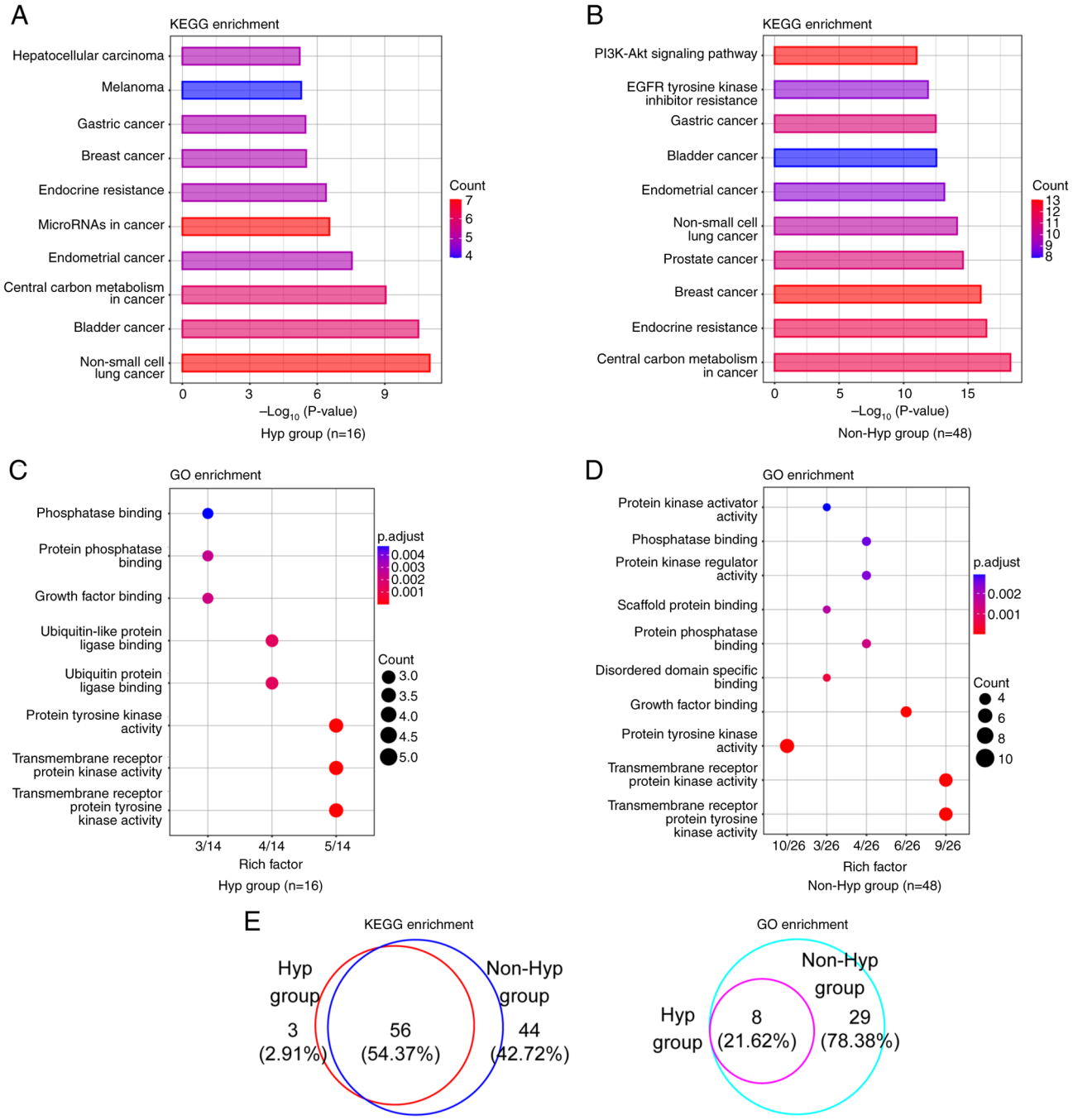

Figure 3. Signaling pathways based on KEGG enrichment analysis and functional terms from GO enrichment analysis. KEGG analysis of patients with non-small cell lung cancer (A) with and (B) without hypertension. GO analysis of patients with non-small cell lung cancer (C) with and (D) without hypertension. The count indicates the frequency of mutant genes enriched in these functional terms. (E) Venn diagrams illustrating KEGG signaling pathways (left) or GO functional terms (right) common in both the Hyp and non-Hyp groups. GO, Gene Ontology; Hyp group, hypertension group; KEGG, Kyoto Encyclopedia of Genes and Genomes; non-Hyp group, non-hypertension group; p.adjust, adjusted P-value.

drinkers (Figs. S4-S8). In addition, Spearman's rank correlation analysis was next performed to examine the correlations among age, BMI, three traditional serum biomarkers and 18 heavy metals in the 14 hypertensive patients. Significant correlations were observed between traditional serum biomarkers and heavy metals, including between CEA and Cr (r=-0.67; P<0.01), between CYFRA21-1 and Zn (r=-0.64; P<0.01), between NSE and As (r=-0.66; P<0.01), between NSE and Ba (r=-0.76; P<0.01), between NSE and Pb (r=0.66; P<0.01) and between NSE and Se (r=0.58; P<0.05). In addition, significant correlations were observed between some of the 18 heavy metals, such as between As and Sr (r=0.54; P<0.05), between Ba and Hg (r=-0.59; P<0.05) and between Cd and Cu (r=0.58; P<0.05; Fig. 5).

Correlations among somatic mutations, demographic and clinical characteristics, traditional serum biomarkers, and heavy metals in patients with NSCLC without hypertension. Compared with patients with *EGFR* mutations, the proportion of *KRAS* mutations (P=0.0192) and smokers (P=0.0265) were

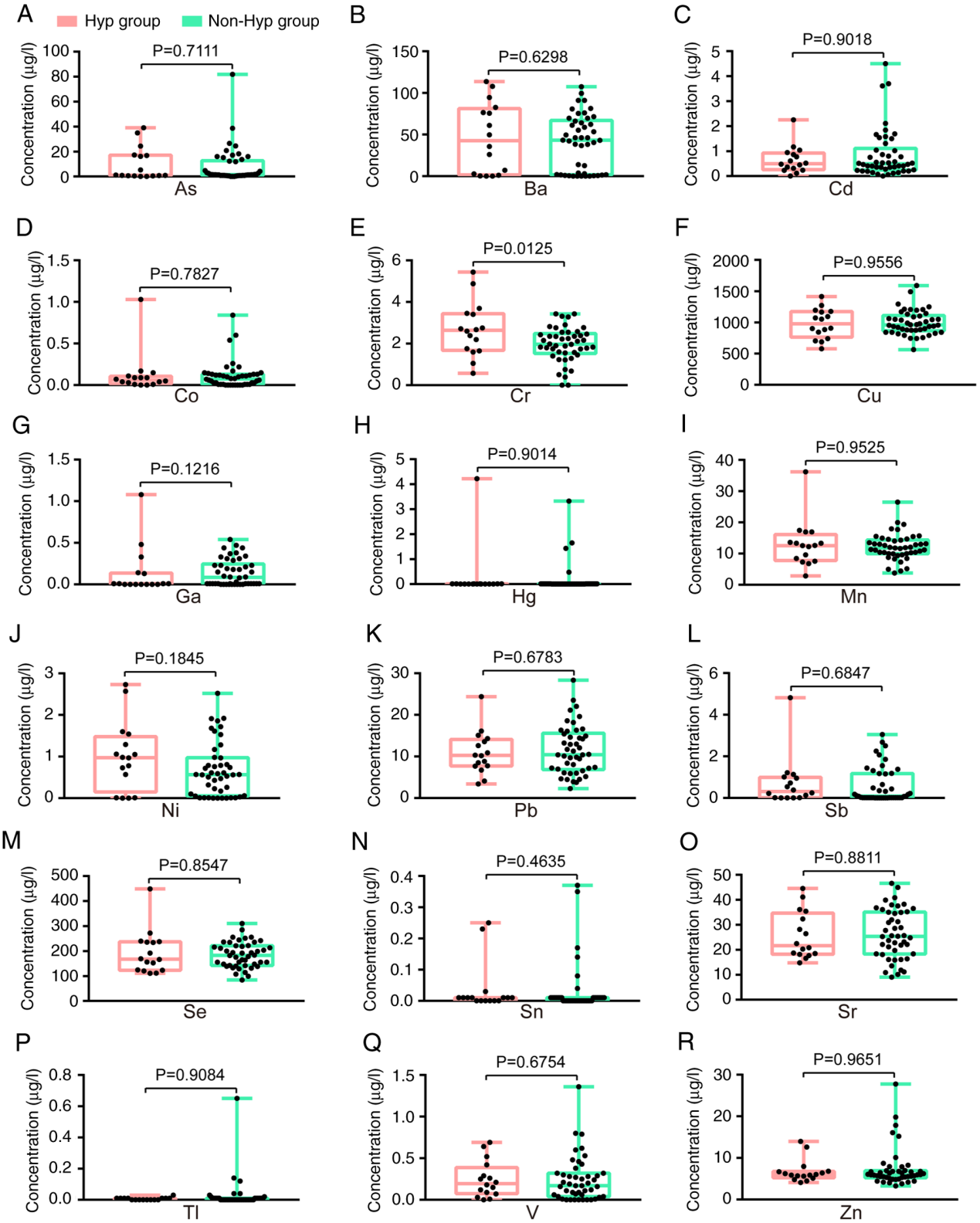

Figure 4. Comparative evaluation of 18 heavy metals in patients with non-small cell lung cancer with and without hypertension. (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cr, (F) Cu, (G) Ga, (H) Hg, (I) Mn, (J) Ni, (K) Pb, (L) Sb, (M) Se, (N) Sn, (O) Sr, (P) Tl, (Q) V and (R) Zn. A two-tailed unpaired t-test was applied for Cr, Pb and Sr, whilst a two-tailed Mann-Whitney U test was used for the remaining 15 heavy metals. As, arsenic; Ba, barium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; Ga, gallium; Hg, mercury; Hyp group, hypertension group; Mn, manganese; Ni, nickel; non-Hyp group, non-hypertension group; Pb, lead; Sb, antimony; Se, selenium; Sn, stannum; Sr, strontium; Tl, thallium; V, vanadium; Zn, zinc.

significantly increased in patients without *EGFR* mutations (Fig. S9; Table SIX). The proportion of women in patients with *TP53* mutations was significantly higher than in patients without *TP53* mutations (P=0.0300) (Fig. S9; Table SIX). In addition, a significant decrease of Zn was observed in patients with *EGFR* mutations in comparison with that in

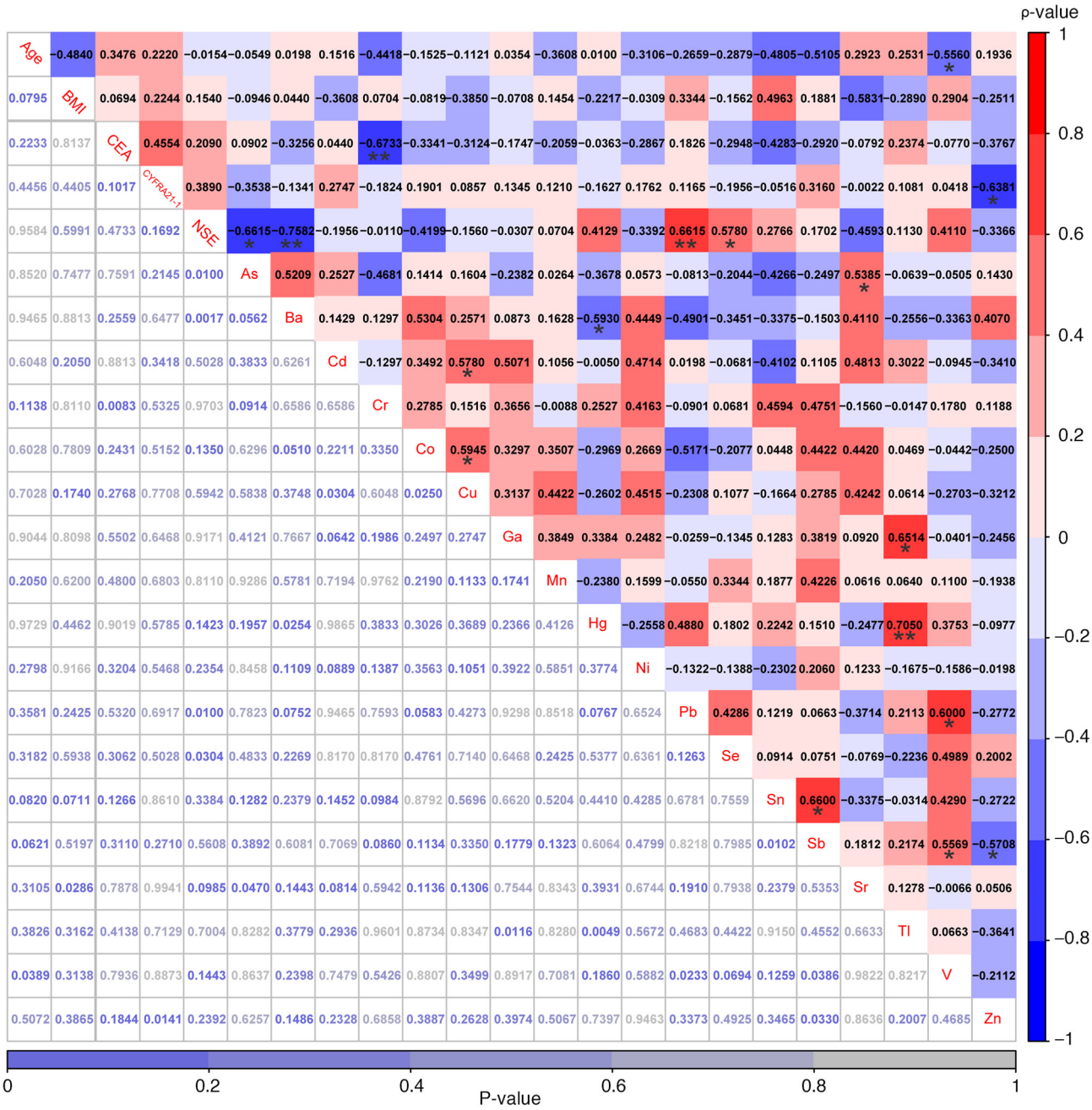

Figure 5. Correlations among age, BMI, three conventional serum biomarkers and 18 heavy metals were explored in patients with non-small cell lung cancer with hypertension. The values highlighted in various colors are the correlation coefficients, with the scale on the right. The values at the bottom are the P-values. *P<0.05 and **P<0.01. As, arsenic; Ba, barium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; CYFRA21-1, cytokeratin-19 fragments; Ga, gallium; Hg, mercury; Mn, manganese; Ni, nickel; NSE, neuron-specific enolase; Pb, lead; Sb, antimony; Se, selenium; Sn, stannum; Sr, strontium; Tl, thallium; V, vanadium; Zn, zinc.

patients without *EGFR* mutations (P=0.0280) according to the two-tailed unpaired t-test (Fig. S10; Table SIX). Compared with patients without *TP53* mutations, patients with *TP53* mutations exhibited a significantly lower level of As and significantly higher levels of Cu and CEA (Fig. S11). The serum levels of Co, Cr and Ni in patients with *KRAS* mutations were significantly higher than those in patients without *KRAS* mutations (Fig. S12). Compared with female patients, male patients exhibited significantly lower levels of Cu and Mn as well as significantly higher levels of Pb and Sn (Fig. S13). Compared with non-smokers, smokers exhibited a significantly lower level of Mn, and significantly higher levels of As, Cd, Pb and Sr (Fig. S14). Compared with non-drinkers, drinkers exhibited significantly lower levels of Ga and Mn, and a significantly higher level of Pb (Fig. S15). Spearman's rank correlation analysis was next performed to examine the correlations among age, BMI, three traditional serum biomarkers and 18 heavy metals in 42 patients without hypertension. BMI was found to be weakly negatively correlated with the serum concentrations of Cu (r=-0.3227; P=0.0371), but no significant correlations were observed between age and other factors (Fig. 6; Table SIX). Furthermore, CYFRA21-1 was significantly positively correlated with NSE (r=0.60; P<0.001), weakly but positively correlated with As (r=0.35; P<0.05) and

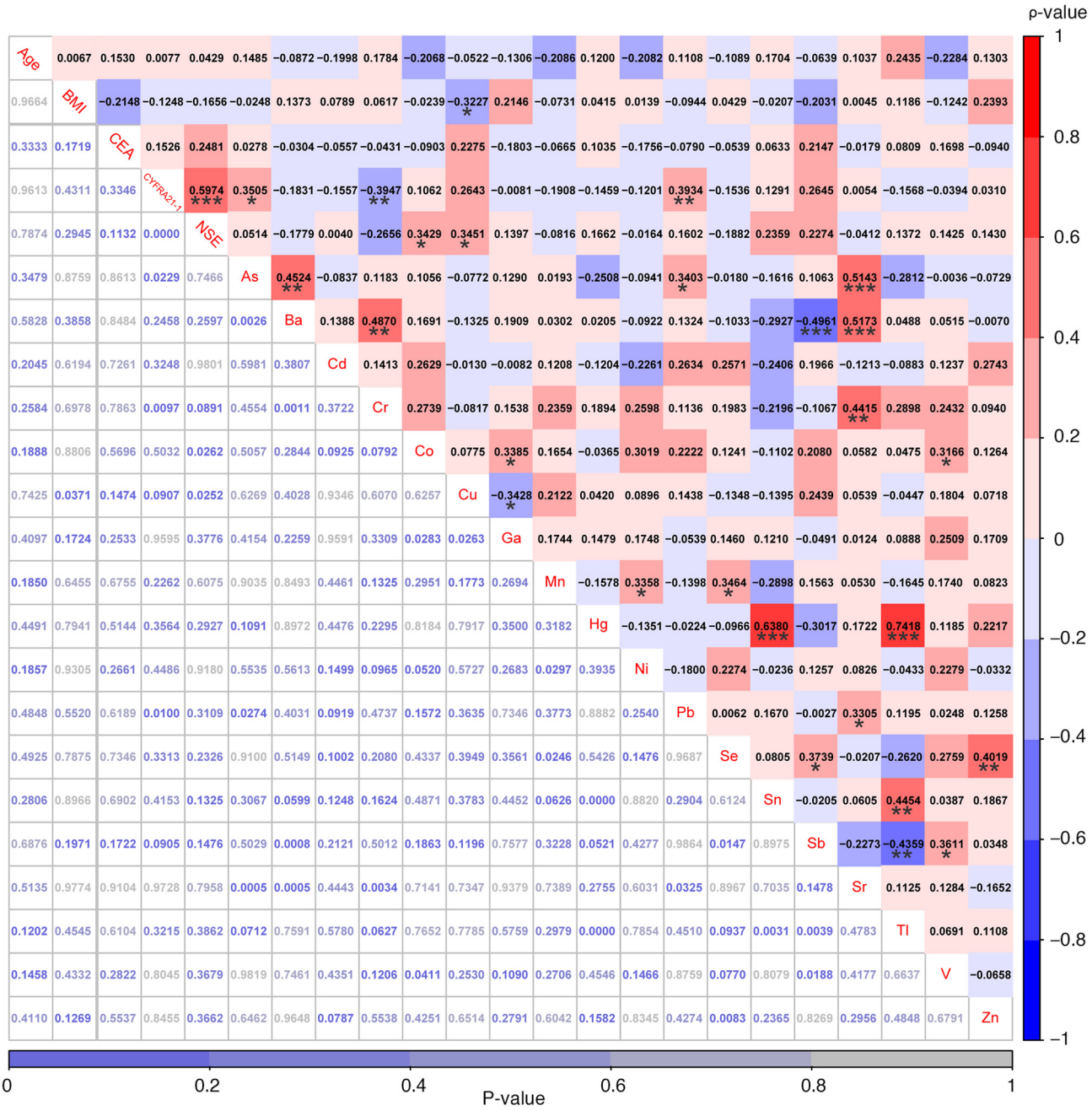

Figure 6. Correlations among age, BMI, three conventional serum biomarkers and 18 heavy metals were examined in patients with non-small cell lung cancer without hypertension. The values highlighted in various colors are the correlation coefficients, with the scale on the right. The values at the bottom are the P-values. *P<0.05, **P<0.01 and ***P<0.001. As, arsenic; Ba, barium; Cd, cadmium; Co, cobalt; Cr, chromium; Cu, copper; CYFRA21-1, cytokeratin-19 fragments; Ga, gallium; Hg, mercury; Mn, manganese; Ni, nickel; NSE, neuron-specific enolase; Pb, lead; Sb, antimony; Se, selenium; Sn, stannum; Sr, strontium; Tl, thallium; V, vanadium; Zn, zinc.

Pb (r=0.39; P<0.01), whilst being negatively correlated with Cr (r=-0.39; P<0.01), differing from only a significant negative correlation between CYFRA21-1 and Zn observed in patients with hypertension. Among the 18 heavy metals, significant correlations were found in Fig. 6, such as between As and Sr (r=0.51; P<0.001), between Ba and Sr (r=0.52; P<0.001), and between Hg and Sn (r=0.64; P<0.001; Fig. 6).

Discussion

Over the past decades, there has been notable improvements in the prognosis and survival of patients with NSCLC (47,48). However, this progress has come with a new set of challenges, since patients, particularly patients with preexisting high blood pressure, the elderly and those with a high body mass index, now face lasting cardiovascular ramifications associated with targeted agents (49-51). Among cancer survivors, CVD stands out as a prominent contributor to morbidity and mortality, ranking second after cancer recurrence (50). This evolving landscape of NSCLC treatment outcomes underscores the importance of addressing long-term cardiovascular implications, emphasizing the necessity for tailored strategies, especially for individuals with specific risk factors.

In the present study, 64 patients with primary NSCLC were recruited, including 16 hypertensive patients and 48 non-hypertensive patients. No significant differences were found regarding the distribution of sex, *EGFR* mutation status, *TP53* mutation status, pulmonary infection history, smoking history, drinking history, cancer staging, presence of blood vessel invasion, nerve invasion, distant metastasis status, CEA levels, CYFRA21-1 levels and NSE levels between patients with and without hypertension, with the only significant difference being the incidence of other chronic diseases (including coronary heart disease, diabetes, atrial fibrillation, Parkinson's disease and chronic gastritis). A total of 47 somatic mutations involving 14 mutant genes were detected in all 16 hypertensive patients (100.00%), whilst 113 somatic mutations involving 26 mutant genes were found in 45 out of the 48 non-hypertensive patients (93.75%). Differences in genomic alterations, somatic interactions, key signaling pathways and enriched biological functional categories were identified between these two groups. Furthermore, the serum concentrations of Cr were significantly elevated in hypertensive patients compared with those in non-hypertensive patients. Finally, significant negative correlations were observed between Cr and CEA, between CYFRA21-1 and Zn, and between NSE and As in patients with hypertension, which were not observed in patients without hypertension. This suggests the existence of differences in the interactive profiles among somatic mutations, traditional serum biomarkers and heavy metals between the Hyp group and the non-Hyp group. To the best of our knowledge, the present study was the first to identify these significant negative correlations between Cr and CEA, between CYFRA21-1 and Zn, and between NSE and As in patients with NSCLC and hypertension in an Eastern Asian cohort.

The prevalence of both lung cancer incidence and mortality exhibits an upward trend in the presence of hypertensive conditions. Previous cohort studies conducted in Finland, Sweden, Norway and Austria indicated an association between hypertension and an elevated risk of lung cancer in both sexes (13,16). Stocks *et al* (13) further suggested that elevated blood pressure levels were associated with an increased risk of mortality, particularly in men rather than women. Conversely, findings from a Dutch study involving 11,075 participants with lung cancer previously revealed that women with hypertensive disorders faced a 2.19-fold increased risk of mortality compared with those without such conditions (17). Additionally, a study in South Korea identified a link between high blood pressure and lung cancer mortality, but subsequent analyses categorized by smoking status implied that the heightened risk was confined to current smokers (18). However, there were no significant differences observed regarding sex distribution and smoking status between hypertensive patients and non-hypertensive patients in the present study. This could be attributed in part to the limited sample size, regional variations or differences in disease status among various studies.

Cigarette smoking is the predominant modifiable risk factor contributing to the onset of lung cancer, which is responsible for ~66% of all lung cancer cases worldwide (52). Nevertheless, the incidence of lung cancer among individuals who have never smoked is also steadily increasing, constituting up to 25% of all diagnosed cases globally (53-55). Several studies have previously indicated a higher propensity for lung adenocarcinoma development in female Chinese patients harboring *EGFR* mutations, particularly among those with no prior history of smoking or alcohol consumption (56-58). In the present study, smoking history was more likely to be observed in men compared with women, which was identified in both the hypertensive and non-hypertensive groups. However, non-smoking patients were more likely to carry *EGFR* mutations than smoking patients, and a significant negative correlation between *EGFR* mutations and smoking history was only observed in patients without hypertension, indicating the difference in interaction characteristics between patients with and without hypertension.

Although inflammation, hypoxia and clonal hematopoiesis are recognized as pivotal players in the intricate relationship between CVD and cancer, the specific mechanisms underpinning the connection between hypertension and an increased incidence of lung cancer remain elusive (59). In the present study, *FBXW7*, *CBR3*, *CDKN2A*, *HRAS*, *SMO* and *UGT1A1* mutations were solely found in hypertensive patients, where the somatic interactions identified in patients with and without hypertension differed markedly. F-Box and WD repeat domain containing 7 (FBXW7), also known as FBW7 or hCDC4, operates as a crucial component within the Skp1-Cdc53/Cullin-F-box-protein complex (60,61). Its function lies in facilitating the phosphorylation-dependent ubiquitination and subsequent proteasome degradation of oncoproteins (61-63). Numerous studies have indicated that the absence of FBXW7 is strongly associated with tumorigenesis, the metastatic spread of tumors, an adverse prognosis and increased resistance to chemotherapy, radiation and immunotherapy, including breast cancer (64,65), cervical cancer (66), colorectal cancer (62,67), gastric cancer (67,68), glioma (69), hepatocellular carcinoma (68), lung cancer (67,70), nasopharyngeal carcinoma (68) and oesophageal squamous cell carcinoma (62). Recently, Wang *et al* (71) reported that the induction of glioma-associated oncogene homolog 1 by the microRNA (miR)-27b-3p/FBXW7/Kruppel-like transcription factor 5 axis may promote pulmonary arterial hypertension in rats. In myeloma fibroblasts, upregulation of miR-27b-3p was found to increase proliferation and resistance to apoptosis via the action of FBXW7 (72). However, to the best of our knowledge, whether miR-27b-3p is involved in the development of NSCLC, particularly in patients with hypertension, remain unclear. These aforementioned results improve our understanding of the pathogenesis of patients with NSCLC with hypertension. In addition, evidence from both experimental animal models and tumor databases (cBioPortal for Cancer Genomics, https://www.cbioportal.org; The Cancer Genome Atlas Program, https://www.cancer.gov/ccg/research/genome-sequencing/tcga) indicated an association between *FBXW7* mutations and a worse prognosis in lung squamous cell carcinoma (62,73), suggesting that FBXW7 may be regarded as an independent prognostic indicator for this disease. To examine this hypothesis, we are collecting prognostic data from patients with NSCLC with various comorbidities, including hypertension, bone metastases and brain metastases, to be analyzed in future studies.

Whilst the precise mechanisms by which heavy metals induce hypertension remain incompletely understood, there is

evidence indicating that blood levels of Cr are independently associated with hypertension (74,75). Prolonged living or working in environments (such as manufacturing shop and maintenance shop of a factory) with elevated Cr exposure is associated with an increased risk of developing hypertension (74,76). Furthermore, plasma concentrations of Cr showed significant correlations with specific clinical markers of pulmonary arterial hypertension severity, including the 6-min walk distance (r=-0.55; P=0.014) and the most recent brain natriuretic peptide level (r=0.463; P=0.039) (77). Consistent with the aforementioned studies, the serum levels of Cr were found to be significantly higher in hypertensive patients compared with those in non-hypertensive patients in the present study. However, contradictory findings also exist. In a previous cross-sectional analysis, serum Cr levels in hypertensive individuals were reported to be significantly lower compared with those in healthy controls (75). In a case-control study, patients with diabetes displayed significant increases in low-density lipoprotein cholesterol, total cholesterol, triglyceride, very low-density lipoprotein cholesterol, insulin, high sensitive C-reactive protein, and homeostasis model assessment of insulin resistance, and a significant decrease in plasma levels of Cr (78). Moreover, a lower Cr level was correlated with high blood pressure, obesity and lipid dysregulation. Overall, large-scale and multi-center studies are required to verify the distributional differences of serum Cr between patients with NSCLC with and without hypertension, which may further refine the management of NSCLC subgroups with different clinical characteristics.

In the present study, a number of limitations must be acknowledged. The NGS data of tumor tissues were obtained from individuals lacking corresponding normal tissues for comparison. This approach was driven by the consideration that a single sample provides sufficient clinically actionable genomic alterations under the appropriate filtering conditions (79-87). It is imperative to acknowledge that opting for the collection of several types of tissues or biopsies from multiple regions incurs higher costs compared with a single-sample approach. The present study also exclusively scrutinized the overall levels of heavy metals in serum, without delving into the repercussions of various forms of a single metal. Additionally, there was a lack of detailed exploration into the sources of exposure, such as soil, water or air, an aspect that falls outside the scope of the present type of study. The assessment of heavy metal concentrations was confined to only serum samples, without corresponding analyses of matched samples (such as urine, hair and nails). Due to the direct reservoirs of certain heavy metals in hair and nails, exploration of the levels of heavy metals in future studies using several different source samples from the same individual is recommended. Furthermore, the sample size in the present study was relatively small, where the demographic diversity of the patients recruited was low. Therefore, the recruitment of several cohorts from multiple institutes from different countries is required to validate the applicability of the findings of the present study.

In conclusion, differences were observed in the presence of genomic mutations, somatic interactions and the serum levels of Cr between patients with NSCLC with and without hypertension in the present study. Furthermore, patients with hypertension exhibited significant negative correlations between Cr and CEA, between CYFRA21-1 and Zn, and between NSE and As, indicating that heavy metals may contribute to the progression of NSCLC in patients with different hypertensive statuses.

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Availability of data and materials

The sequencing data generated in the present study may be found in the National Center for Biotechnology Information BioProject database under accession number PRJNA904839 or at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA904839. The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

XZ, JY and HJ were involved in the conception and design of the study. XZ, HS and YW supplied patient samples, and analyzed and interpreted the clinicopathological data. JY, MW, LJ and HJ carried out data collection and analysis. All authors were involved in writing the manuscript and revising it critically. XZ, JY and HJ confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

For the use of human samples, the present study was approved by the Medical Ethics Committee of The First People's Hospital of Ping Ding Shan (approval no. PYLL20190806; Pingdingshan, China). Written informed consent was obtained from all patients.

Patient consent for publication

All patients provided written consent for the publication of data.

Competing interests

The authors declare that they have no competing interests.

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