A modified approach allows for inquiry of previously alien TEPs in peripheral blood, possibly related to lung cancer progress.

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Abstract

Background
While the PRP (platelet-rich plasma) method was the most widely used in the tumor-educated platelets (TEPs) research, there were still platelets in peripheral blood unextracted which are whose histology and function are currently alien.

Objectives
To prove the unextracted platelets existed, and to explore the correlation of the unextracted platelets with lung cancer progression.

Methods
A second centrifugation step was used to establish a modified approach, which was compared with the PRP method based on the PRR (platelet recovery rate). Sequencing analysis was used to explore the function of unextracted platelets.

Results
The modified approach improved the PRR to 100%, while the PRP method got the PRR at 70%. The PRR was negatively correlated with platelet volume, and positively correlated with platelet counts. Those results indicated that about 30% of platelets with larger sizes were unextracted in the PRP method. The Sequencing analysis revealed that those platelets are abundant in RNA and might be more pertinent to the progression of lung cancer connected with the mitogen-activated protein kinase (MAPK) signaling pathway.

Conclusions
This research raised a modified approach to collect the unextracted platelets omitted by the PRP method in peripheral blood, which provided technical support for TEPs’ application in liquid biopsy. We also proved those alien platelets might play a role in lung cancer progression, which would allow further mechanistic exploration.

Introduction
Platelets were previously thought to be involved in the regulation of hemostasis and thrombosis. However, platelets are now known to play a role in tumor growth and metastasis (1). Since platelets are
activated, aggregated, release platelet-derived chemicals into the bloodstream, and enhance thrombocytosis in tumor settings, the theory of tumor-educated platelets (TEPs) has been suggested (2). Platelets are the most prevalent anucleate cell in the bloodstream, second only to red blood cells, and they engage in bidirectional tumor-platelet interactions, which suggests a wide range of expression profiles and simple enrichment (3). Consequently, the TEPs have caught the interest of researchers as a novel liquid biopsy biomarker.

The noninvasiveness, frequent multiple detection possibilities, and fast reaction times of liquid biopsy have made it more and more popular in recent years. Extraction of the maximum number of platelets from the peripheral blood is the main technical key used in liquid biopsy. The platelet-rich plasma (PRP) method is the most often used extraction technique. However, our previous studies have shown that it only extracts 70% of platelets from normal participants and leaves about 50% of lung cancer patients’ platelets unextracted (4). Based on the results, we assumed that due to the limitation of centrifugation, the PRP method exclusively retrieved platelets from the upper PRP layer, hence unextracted platelets were deposited in the lower layer after centrifugation due to their greater size or the presence of aggregation.

In this work, a modified approach was proposed for extracting the unextracted platelets, which increased the quantity of platelets retrieved. Otherwise, the previous data obtained from platelet extraction and sequencing using the PRP method only reflect platelets in the PRP layer and do not represent all platelets in peripheral blood (5). The unextracted platelets are those whose histology and function are currently unknown, which attracted us. So, sequencing analysis was used to explore the function of unextracted platelets, which revealed those platelets are abundant in RNA and might be more pertinent to the progression of lung cancer connected with the mitogen-activated protein kinase (MAPK) signaling pathway.

**Method**

**Study design**

This prospective, observational study was started from 2020 until 2023 in Sichuan Cancer Hospital, and approved by the medical ethical committee of Sichuan Cancer Hospital (SCCHEC-02-2020-043). As shown in Fig. 1, this research was completed in two parts. In the first part, to reveal that there were unextracted platelets by the PRP method and to modify the PRP method, specimens from 40 healthy individuals were collected. In the second part, 6 specimens from 3 healthy individuals and 3 lung adenocarcinoma (LUAD) patients were collected, and then completed the transcriptome sequencing to reveal the function of the PRP-unextracted platelets. The TEPs datasets GSE 68086 and GSE 89843 established by Myron Best were downloaded from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) (5). The Venn Diagram tool was used to identify specific genes among differentially expressed genes (DEGs) in the PRP platelets, the PRP-unextracted platelets, and the
TEPs datasets of lung cancer patients. These genes may serve to find the cancer-related pathways ignored by the PRP method.

**Study participants**

All the specimens were collected prospectively and analyzed retrospectively. The specimens from participants were collected who underwent physical examination in Sichuan Cancer Hospital, further selected the specimens reflecting healthy individuals. The healthy participants were enrolled if they were Han ethnic group aged between 18 and 79. The participants’ nutritional condition and coagulation needed to be normal. The participants would not be included if they have any existing health issues (comorbidities), are pregnant, or have previously used or are still using alcohol, cigarettes, oral contraceptives, or anti-platelet drugs. Also, the participants with surgery history within 6 months of enrollment, blood donation, or transfusion within 4 months of enrollment would be excluded. If there were aberrant clinical laboratory values, such as platelet count < 100 × 10^9/L or > 300 × 10^9/L, or deficiency of necessary data, the specimens would be excluded either.

And the specimens from lung cancer patients were collected as previously (4). The blood samples were collected and determined at the same time participants were admitted hospital. The individuals consecutively diagnosed with a first occurrence of pulmonary nodules, and then verified as malignancy were enrolled in this study. Thus, the participants should be excluded as follows: 1) patients without completely necessary data, and not confirmed by the surgery or biopsy; 2) patients with other cancers and acute inflammation, or receiving the medicine influencing the platelets; 4) patients who received platelet or blood transfusion in 4 months(6).

**Platelet collection using the PRP method**

Fresh whole blood specimens (2 mL/person) from participants were drawn into EDTA-K2 tubes (Co.Sanli, Liuyang, Hunan, CHN). In fresh whole blood specimens, bPLT (platelet count in whole blood samples), and HCT (hematocrit in whole blood samples) were determined with a Mindray cell counter BC-6600 (Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, CHN). Then, 1.5 ml EDTA anticoagulated blood was added to 2 ml EP tubes, and centrifuged at 120*g for 20 minutes to separate supernate which was PRP (Co., Ltd.Shuke, Chengdu, Sichuan, CHN). PRP was removed to a new EP tube gently, while the precipitation was red blood cell layer (RBCL). The pPLT (platelet count in PRP samples) was determined with a Mindray coulter counter 6600 immediately. After that, PRP was centrifuged at 360*g for 20 minutes, so that precipitation was platelets while supernatants were platelet-poor plasma (PPP). The platelets in the precipitation collected at this step were the uPLT (platelets from the upper PRP layer) (shown in Fig. 2A). As we reported before, PRR=$\frac{pPLT \times v \times (1-HCT)}{bPLT \times v} \times 100\%$(4). To prevent platelet activation and aggregation, all the operations needed to be gentle.

**Platelet collection using the modified approach**
To improve the PRR, a re-centrifuge was used to gather the remained platelets in the RBCL. The RBCL was mixed with PPP and re-centrifuged at 120*g for 20 minutes to separate PRP2 (PRP separated in the 2nd centrifugation). Same as before, PRP2 was removed to a new either, while the pPLT2 (platelet count in PRP2 samples) was determined with a blood cell counter. Platelets were also collected after that PRP was centrifuged at 360*g for 20 minutes, which were the dPLT (platelets from the down red blood cell layer). Thus, uPLT plus dPLT was the final platelets that we could enrich (shown in Fig. 2C). And the new PRR (nPRR) could be calculated as the formula, nPRR = \( \frac{pPLT \times v \times (1 - HCT) + pPLT2 \times v \times (1 - HCT)}{bPLT \times v} \times 100\% \).

Transcriptome sequencing of uPLT and dPLT

The specimens (4mL/person) from lung cancer patients and healthy individuals were collected, and then the uPLT and dPLT were further separated from the two groups of specimens respectively as above. Total RNAs of uPLT and dPLT were extracted using the TRizol method. The quantity and quality of RNA is typically measured using Qubit3.0 (Life Invitrogen Qubit® 3.0), Nanodrop (NanoDrop™ One/OneC), and Agilent Bioanalyzer (Agilent 4200 TapeStation). Following RNA qualification, the next step was the creation of an RNA-Seq library, which was performed with AMPure XP beads. After RNA-Seq was qualified, library Illumina PE150 sequencing was performed after pooling different libraries according to the requirements of effective concentration and target data volume. RNA-Seq data was analyzed by FASTQ-format files, and then paired-end clean reads were aligned to the reference genome using HISAT2 v2.1.0 (hierarchical indexing for spliced alignment of transcripts). Feature count was used to count the reads numbers mapped to each gene. Then FPKM and TPM of each gene was calculated based on the length of the gene and the read count mapped to this gene.

Exploring specific genes expressed in dPLT

Subsequently, differential gene analysis was performed using the DESeq2 R package (version 1.40.2) applying the TEPs datasets and sequencing data to compare gene expression profile data from non-small-cell lung cancer (NSCLC) patients with healthy controls to identify DEGs for NSCLC (7). The criteria for screening disease-related DEGs were with p-values < 0.05 and |logFC| > 2. Candidate genes with differential expression specifically expressed in dPLT were identified by the Venn diagram R package. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and HALLMARK enrichment analysis and visualization of differentially expressed genes were implemented by the metascape (https://metascape.org) (8). The GEPIA platform (http://gepia.cancer-pku.cn/) was used to analyze the expression of candidate genes between normal and LUAD tissues(9), while the UALCAN platform (https://ualcan.path.uab.edu/index.html) analyzed expression levels of candidate genes in subgroups of LUAD patients with different tumor stage (10). The TPM value was used as a reference for the gene expression level. The STRING database (https://string-db.org/) was used to explore the protein-protein interactions among candidate genes(11).

Quality control

The platelets collection in this research was performed by the same operator, which could minimize the operating error caused by different operators. The quality control of Mindray coulter counter assessed
using whole blood controls for cell counting, while External Quality Assessment results of the laboratory were 91 to 100 points. The specific centrifuge was used to avoid experimental error, which was also calibrated half a year. RNA purity (OD260/280, OD260/230 ratio) was detected with NanoDrop™ One/OneC, precise quantification was detected with Life Invitrogen Qubit® 3.0, and RNA integrity was detected with Agilent 4200 TapeStation system (RIN value). Precise quantification of library concentration was performed with Kapa qPCR, while accurate detection of library fragment sizes was performed with Agilent 4200 TapeStation, which ensured the quality of the RNA-Seq library.

### Statistical analysis

Statistical analysis was conducted with R (http://www.R-project.org). Shapiro-Wilk tests were used to assess the normality of the distribution. The data display consisted of the median and Interquartile range (IQR). Wilcoxon test was used to test for non-normal distributions. The p-values < 0.05 were considered statistically significant.

### Results

#### The characters of all participants

In part 1, 22 female and 18 male participants were enrolled with normal platelet features (Table 1). In part 2, there were 3 healthy participants and 3 patients with LUAD who were age and gender-matched (P > 0.05) (Table 1). Similar to the previous research, the trends could be seen that the platelet counts (bPLT) in whole blood samples was lower, and mean platelet volume (bMPV) in whole blood samples was higher in malignant participants.

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The comparison of the PRR method and the modified approach

For the PRP method, the platelet was enriched in the upper plasma after the first centrifugation, which could be separated by precipitating PRP, while there was still a part of the platelets remaining in the RBCL (Fig. 2A). In the modified approach, part of the platelets in RBCL could be mixed with PRP further to be separated from PRP2 (Fig. 2C). Based on that, pPLT and pMPV were used to reflect the platelet count and volume in PRP, while pPLT2 were used to reflect the platelet number in PRP2 implying platelet number in RBCL partly. As shown in Fig. 2B, only 72% of platelets (PRR1) could be segregated using the PRP method, and another 28% of platelets could be segregated in the PRP2 sample (PRR1), with a jump to 100% in PRR (P < 0.001). The platelet counts of the high-level PRR2 group (239.50, 205.50-303.25, ×10^9/L) decreased than that of the low-level PRR2 group (202,183.5-238.5, ×10^9/L) (P = 0.02), whereas the MPV of high-level PRR2 group (9.8, 9.275–10.225, fL) showed a decrease than that of low-level PRR2 group (10.2,9.875–10.625, fL) (P < 0.01) (Fig. 2D, F). The correlation results also showed an inverse correlation between MPV and PRR2, while a positive correlation was observed between platelet counts and PRR2(Fig. 2E). Supplementary Table 1 contains comprehensive information on the study's findings as well as the fundamental details of the Part 1 participants. Supplementary Fig. 1 displays the preliminary methodological comparisons between the PRP and the modified method.

The comparison of RNAs extracted from uPLT and dPLT

The RNA concentration was compared at first, which showed more RNAs contained in the dPLT (Fig. 3A, B, C). In the malignant individuals, the datasets of uPLT ranged in total sequence depth with a median value of 34.01 million (IQR:32.60–37.74 million), while that of dPLT ranged with a median of 35.18 (IQR: 27.40- 38.27 million). The casual genome coverage was achieved in uPLT analysis rather than dPLT, indicating that more unknown transcript information remained in the dPLT. Then, in both the malignant and healthy groups, the attribution to gene segments was investigated between uPLT and dPLT. As anticipated, the absolute number of uPLT in the exonic region (Exon) and the intronic region (Intron) was relatively high, but in both the malignant and healthy groups, the number ratio in Intron was lower than that of dPLT. Comparably, in InterGenic, uPLT was more common in terms of absolute number, but after normalization, the number ratio was smaller than that of dPLT (Fig. 3D, E). This portion of the data also implies that more functional and unidentified non-coding areas remained in dPLT. Supplementary Table 2 displays the precise expression analysis results, and Supplementary Table 3 displays the subgroup statistics for the expression analysis.

The DEG analysis of uPLT and dPLT

After analyzing, a total of 1677 DEGs were found in dPLT, of which 1350 (80.5%) were up-regulated and 327 (19.4%) were down-regulated (Fig. 4A). A total of 634 DEGs, comprising 213 (33.6%) up-regulated and 421 (66.4%) down-regulated genes, were found in uPLT (Fig. 4B). Combining the data from GSE 68086 and GSE 89843, 811 DEGs were found within 802 genes that were up-regulated (98.89%) and 9
genes that were down-regulated (1.11%) (Fig. 4C). The top 1000 genes in uPLT, dPLT, and GSE were sorted according to |logFC| values and subjected to Venn diagram analysis. This revealed that 936 genes were exclusively DEGs in dPLT (Fig. 4D, Supplementary Table 4). Hallmark and KEGG enrichment revealed that the 936 DEGs were primarily related to cancer pathways, including the tumor necrosis factor-alpha nuclear factor kappa B subunit (NFKB-TNFA) and MAPK signaling network (Fig. 4E, F).

**The exclusively DEGs in dPLT are associated with LUAD progression**

According to earlier research, there is a positive correlation between tumor advancement with MPV and platelet counts (12). Thus, correlation analysis was used to identify the genes linked to MPV and platelet count, which showed results that platelet counts were favorably connected with the expression of FAM204A, ACSL1, FAM120A, NDUFA4, HIST1H2BC, and GABARAPL2, while MPV was negatively correlated with ACSL1, FAM120A, HIST1H2BC, and GABARAPL2 (Fig. 5A). As shown in the heatmap (Fig. 5B) and Chord Diagram (Fig. 5C), FGFR1, and JUN were upregulated in LUAD. The String protein interaction study of four genes, ACSL1, FAM120A, HIST1H2BC, and GABARAPL2, with 26 genes enriched for the MAPK pathway revealed that HIST1H2BC may interact with MYC, JUN, and other MAPK signaling pathways (Fig. 5D). Further, the expression of HIST1H2BC, JUN, FGFR1, and other MAPK-enriched genes in TCGA were explored, while HIST1H2BC was highly expressed in LUAD patient tissues (Fig. 5E), whereas FGFR1 and JUN in the MAPK signaling pathway were lowly expressed in LUAD patient tissues (P < 0.05) (Fig. 6F, G, supplementary Fig. 2). We analyzed the TPMs of particular DEGs, in the dPLT, which discovered that HIST1H2BC were lowly expressed in the dPLT (Fig. 5H) (P = 0.064) and that JUN and FGFR1 were highly expressed in the dPLT of LUAD patients (Fig. 5I, J).

**Discussion**

Since the concept of TEPs was developed, the collection of platelets from peripheral blood was important in clinical and basic science studies. As reported, the widely used method for collecting platelets from peripheral blood was the PRP method, while the TEPs sequenced and uploaded in GSE datasets were also collected with the PRP method (5)(13). PRP method was a centrifugation method, which distinguished peripheral blood into a red blood cell layer and a plasma layer containing most of the platelets (14). The PRP technique was the basis for earlier platelet research, and the platelets found in the erythrocyte layer were alien platelets that had not yet been removed, analyzed, or sequenced. In this research, to collect the alien platelets, a second centrifugation step was used to establish a modified approach, which could collect all platelets from peripheral blood.

As we have reported PRR to present the platelets collection rate from whole blood, our modified approach has improved PRR almost to 100%. This shows that our method is capable of obtaining almost all platelets in peripheral blood, and it also suggests that this fraction of platelets cannot be extracted using the PRP method. Our modified approach offers technical assistance for the application of alien platelets for cancer early diagnosis. However, the relationship between PRR2 with bMPV and bPLT
suggests that platelets with larger size and number are more likely to be deposited in the RBCL layer. As previous research proved that larger size platelets might correlate with lung cancer progression, the alien platelets attracted us for their possible role in cancer progression (12)(15)(16). Interestingly, the platelets in the erythrocyte layer retain not only a greater number of functional and unknown noncoding areas but also an amount of totally different DEGs. This implies that dPLTs may have further unidentified roles in the development of tumors in addition to being an entirely distinct type of platelet from those found in earlier research, which were exactly alien platelets.

Further to investigate the reasons behind the large platelets that are deposited in erythrocytes and determine whether any of these may be connected to tumor stimulation, we first performed a correlation analysis to identify potential genes associated with large platelets. The four genes, ACSL1, FAM120A, HIST1H2BC, and GABARAPL2, had a negative correlation with MPV in dPLT, might be essential for the development of large platelets. What mechanism do tumor cells use to change the expression of these four genes to cause platelets to enlarge, given that these genes are not important in normal and uPLT populations? From the perspective of tumor progression, we discovered that the dPLT DEGs were potentially enriched in the tumor-associated MAPK pathway. Subsequently, the String platform was used to investigate whether the four genes ACSL1, FAM120A, HIST1H2BC, and GABARAPL2 might be connected to the activation of the MAPK pathway in the tumor cells via specific pathways, resulting in modifications to the expression of the genes and the production of large, easily activated platelets. Consequently, HIST1H2BC may interact with MYC, JUN, and other proteins in the MAPK signaling pathway. Hence, we were interested in learning more about the potential relationship between platelet HIST1H2BC and the MAPK pathway in tumor cells. It was observed that HIST1H2BC was highly expressed in LUAD patient tissues, whereas JUN and FGFR1 in the MAPK signaling pathway were lowly expressed in LUAD patient tissues. Conversely, HIST1H2BC was lowly expressed in peripheral blood dPLT, whereas JUN and FGFR1 were highly expressed. Following searching through the literature, we discovered that tumor cells may intercept platelets in the tumor microenvironment in quest of proteins, nucleic acids, and other substances (17)(18). Based on this, we conjectured that platelets exhibiting elevated HIST1H2BC expression could be seized in LUAD tumor tissues, resulting in elevated HIST1H2BC expression in tumor tissues concurrent with platelet HIST1H2BC depletion. The hijacked HIST1H2BC may further activate the tumor cells' MAPK signaling pathway while also transmitting to peripheral platelets, causing the latter to highly express genes linked to the MAPK signaling pathway and become readily activated. This allows the peripheral platelets to enter peripheral blood and form a significant volume of dPLT. This precisely implies that alien platelets might come into direct contact with tumor cells and then be the source of dPLT, which is more valuable for diagnostic purposes. Next vivo tests will confirm this notion, providing a theoretical basis for the use of dPLT for early LUAD detection (supplementary Fig. 3).

Limitation

Due to the lack of a standard method for extracting peripheral blood platelets, its consistency was only assessed by comparing it with the commonly used PRP method, following which a more comprehensive
methodological evaluation through standard samples would be established. In this investigation, a direct comparison of the MPV in dPLT and uPLT was absent to dependably affirm that the volume of dPLT is more than that of uPLT, due to processes like centrifugation, transferring plasma, and mixing blood activating platelets further to influence the results of pMPV. The manipulating restrictions resulted in PRR2 greater than 100%. Not all the PRP might have been pipetted after the first centrifugation, and the remained PRP might have stayed with red blood cells and mixed with PPP to participate in the second centrifugation. Furthermore, our hypothesis has yet to be validated by additional clinical and basic studies, which we will do in the future.

Conclusion

Consequently, the current work verifies the existence of a portion of TEPs that are considerably larger which are alien platelets in earlier research. These alien platelets may be more pertinent to the progression of tumors, in addition to offering a novel technique for increasing the number of platelets in peripheral blood. Through the implementation of our novel approach, peripheral blood TEPs will be more thoroughly obtained, thereby giving a comprehensive reflection of the interaction between platelets and tumors in tumor patients as well as a research foundation for further multi-omics investigations of TEPs.

Abbreviations

TEPs
Tumor-educated platelets
PRP
platelet-rich plasma
DEGs
differentially expressed genes
NSCLC
non-small-cell lung cancer
TNFA-NFKB
tumor necrosis factor-α-nuclear factor kappa B subunit
MAPK
mitogen-activated protein kinase
LUAD
lung adenocarcinoma
GEO
Gene Expression Omnibus
GSE
GEO Series
bPLT
platelet count in whole blood samples
HCT
hematocrit in whole blood samples
RBCL
red blood cell layer
pPLT
platelet count in PRP samples
PPP
platelet-poor plasma
uPLT
platelets from the up layer
PRR
platelet recovery rate
dPLT
platelets from the down layer
nPRR
new PRR
FPKM
Fragments Per Kilobase Million
TPM
Transcripts Per Kilobase Million
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
IQR
Interquartile range
bMPV
mean platelet volume in whole blood samples
bPDW
platelet distribution width in whole blood samples
Tes
Transcription termination sites
UTR3
3’ Untranslated Regions
UTR5
5’ Untranslated Regions
Intron
the intronic region
InterGenic
the intergenic region
ExonALL
Declarations

Ethics approval and consent to participate
This research was approved by the medical ethical committee of Sichuan Cancer Hospital (SCCHEC-02-2020-043).

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Funding
Sichuan Province Medical Science Research Youth Innovation Project (Q22076).

Author Contribution
Zu and Luo designed this work. Zeng, Deng, Yin, and Xu collected the samples and analyzed them. Zu and Zhang analyzed and visualized the data. Zu wrote the original draft. Han, Li and Wang corrected the original draft. All authors reviewed the manuscript.

Acknowledgement
We would like to express our sincere gratitude to all individuals and organizations who have contributed to the success of this research project. We thank everyone involved for their commitment and support, which have been essential to the completion of this research.
Availability of data and materials

Part of the data generated or analyzed during this study is included in this published article [and its supplementary information files]. The other data used and analyzed during the current study are available from the corresponding author on reasonable request.

References


Figures
This research consists of two parts. It was established in the first Part that while all platelets could be extracted from peripheral blood using the modified approach, not all platelets could be extracted from peripheral blood using the PRP method. The second part investigated the function of PRP-unextracted platelets in lung cancer patients extracted by the modified approach.
Figure 2

The operation and comparison of two methods. (A) The procedure of the PRP method. (B) The PRR comparison of the PRP method and the modified approach. (C) The procedure of the modified approach. (D) The platelet counts comparison of the high-level PRR2 group and the low-level PRR2 group. (E) The Correlation heat map of the PRR and the platelet features. (F) The MPV comparison of the high-level PRR2 group and the low-level PRR2 group.
Figure 3

Expression analysis results of dPLT and uPLT. (A) The RNA concentration comparison between dPLT and uPLT within healthy participants. (B) The RNA concentration comparison between dPLT and uPLT within LUAD participants. (C) The RNA concentration comparison between dPLT and uPLT within all participants. (D, E) The genome annotation data was compared with the positions of the sequence alignment results on the reference genome, and the number of reads in the 1500 bp region above and
below the transcriptional start and termination (Tes), the genomic UTR region (UTR3 and UTR5), the
intrinsic region (Intron), the intergenic region (InterGenic), the CDS region, and the exonic region
(ExonALL) were counted, respectively in healthy participants (D) and LUAD participants (E).

Figure 4
The DEG analysis of uPLT and dPLT. (A) The volcano plot of DEGs in the dPLT. (B) The volcano plot of
DEGs in the uPLT. (C) The volcano plot of DEGs in the GSE dataset. (D) Venn diagram of the top 1000
genes in the uPLT, dPLT, and GSE dataset. (E) The Hallmark enrichment analysis of the exclusively DEGs in the dPLT. (F) The KEGG enrichment analysis of the exclusively DEGs in the dPLT.

Figure 5

The exploration of the tumorous function of the dPLTs. (A) The correlation heatmap of the gene expression and blood routine test features. (B) The heatmap of the expression of genes related to LUAD progression. (C) The Chord Diagram maps the relationship between the genes and pathways. (D) The
possible relationship between MPV-related genes and MAPK pathways enriched genes. (E-G) The expression (TPM) of HIST1H2BC (E), JUN (F), and FGFR1 (G) in healthy and LUAD individuals based on GEPIA platform. (H-J) The expression (TPM) of HIST1H2BC (K), JUN (L), and FGFR1 (M) in healthy and malignant individuals is based on our transcript sequencing results.

**Supplementary Files**

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

- sfig1.pdf
- sfig2.pdf
- sfig3.pdf
- supplementarytable1.csv
- supplementaltable2.xlsx
- supplementaltable3.xlsx
- supplementaltable4.csv
- Supplementary.docx