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ORIGINAL ARTICLE – CANCER RESEARCH

A three-platelet mRNA set: *MAX, MTURN* and *HLA-B* as biomarker for lung cancer

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Abstract

Background During the development of tumors, tumors "educate" platelets causing changes in their mRNAs expression profiles and phenotypes, thereby, tumor-educated platelet (TEP) mRNA profile has the potential to diagnose lung cancer. The current study aimed to examine whether TEPs might be a potential biomarker for lung cancer diagnostics.

Methods Platelet precipitation was obtained by low-speed centrifugation and subjected to Trizol for total RNA extraction. Platelet *MAX, MTURN,* and *HLA-B* mRNA were selected by microarray, validated by qPCR, and analyzed combined with related clinical factors.

Results Our results showed that a three-platelet mRNA set: *MAX, MTURN*, and *HLA-B* was significantly up-regulated in lung cancer patients as well as in early-stage lung cancer patients compared with those from healthy donors, the area under the curve (AUC) was 0.734, 0.787, respectively, among which platelet *MTURN* mRNA processed a dramatically high diagnostic efficiency in female patients with lung cancer, its AUC for female was 0.825. More importantly, the three-platelet mRNA set: *MAX*, *MTURN*, and *HLA-B* was associated with chemotherapeutic effect, low mRNA expression of this three-platelet set was correlated with "favorable" first chemotherapy response.

Conclusions A three-platelet mRNA set: *MAX, MTURN and HLA-B* enables blood-based lung cancer diagnosis and chemotherapy response prediction.

Keywords TEPs · Platelet mRNA · *MAX* · *MTURN* · *HLA-B* · *UQCRH* · Biomarker

Introduction

Lung cancer is one of the most common malignant tumors in the world with morbidity and mortality. Its onset is concealed, and early clinical manifestations are lack of specificity (Torre et al. 2016; Zou et al. 2016). Despite the continuous improvements in surgical resection, irradiation, chemotherapy and targeted therapy, lung cancer patients are extremely vulnerable to relapse and mortality, since most patients have had local or distant metastasis at the time of diagnosis (Jemal et al. 2006). Hence, a sensitive, specific, blood-based, noninvasive test for detection and prediction of therapy response is conducive to outcome of lung cancer patients.

Platelets are anucleate blood cells (2–4 µm in diameter) with multiple functions, and a short lifespan (circulating in blood for 7–10 days in humans) (Quach et al. 2018) and the second in the number only to red blood cells (Haemmerle et al. 2018). They are derived from megakaryocytes in bone marrow, or even in the lung by intravascular

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megakaryocytes in vivo (Lefrancais et al. 2017), and traditionally known for their role in hemostasis and initiation of wound healing (Leslie 2010). More recently, platelets have emerged as central players in the systemic and local responses to tumor growth. Thrombocytosis is present in more than 30% of patients with solid malignancies and correlates with worsened patient survival (Haemmerle et al. 2018). Tumor-derived interleukin-6 (IL-6) stimulated thrombopoietin (TPO) production, thus stimulating megakaryopoiesis and thrombocytosis (Stone et al. 2012). In fact, increased numbers of activated platelets also promote further tumor growth and metastasis (Guo et al. 2012).Tumor cells give rise to platelet aggregation, which results in the formation of platelet–tumor cell complexes, thus significantly reducing the immunogenicity of tumor cells to escape epidemic surveillance, and reducing the impact of blood turbulence and the damage of shear stress in blood flow on tumor cells (Goubran et al. 2013, 2014; Buergy et al. 2012).

Platelets have long been considered as a potential diagnostic tool in cancer. They are easily isolated and counted in blood tests. Several studies have shown that platelet count (Matowicka-Karna et al. 2013), size (Wang et al. 2015) as well as protein markers, such as P-selectin (Laubli and Borsig 2010) already harbor potential clinically relevant information and are used for blood-based cancer diagnostics and prognostics. Platelets can interact with cancer cells and educated via transfer of tumor-associated biomolecules, termed as "tumor educated platelets (TEPs)" (Best et al. 2018), whose proteome content and RNA profile were changed significantly. The changes of platelets profile represent a massive, concentrated biorepository of tumor-derived and bioactive molecules, indicating the potential of platelets as specific biomarkers for cancer. It has been reported that analysis in patients with multiple myeloma identifies differential TEP RNA profiles between healthy individuals and patients with smoldering multiple myeloma, which is activated with disease progression via IL-1β upregulation (Takagi et al. 2018). Besides, TEP RNA profiles precisely pinpoints the primary origin of pan-cancer, as well as predicted the oncogenic status of many tumors including *MET* or *HER2* positivity and the existence of *KRAS*, *EGFR*, or *PIK3CA* mutations (Best et al. 2015). Consequently, these findings suggest TEP RNA profiles as cancer biomarkers.

In our study, we firstly screened the differential TEP mRNAs between lung cancer patients and healthy donors using microarray and then validated them in a larger cohort via quantitative PCR, based on which a three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* was selected, followed by the analysis for their diagnostics efficiency and predicting chemotherapy response assessment, thus providing the evidence this three-platelet mRNA set as a biomarker for lung cancer.

Methods and materials

Patients and healthy donors

Total 225 lung cancer patients and 185 healthy donors admitted to Shandong Cancer Hospital and Institute from February 2018 to June 2018 were enrolled in this study. Lung cancer patients were diagnosed by the combination of clinical, pathological, radiological approaches, and the tumor stage was determined according to the eighth edition of the lung cancer TNM staging standards formulated by *IASLC*. All patients did not receive any anti-tumor treatment before peripheral blood collection, or suffer any other endocrine, immune, or metabolic diseases. The healthy donors did not present any disease. Informed consent was obtained for all individuals.

Wright–Giemsa staining

The platelets were separated from the whole blood by twice $120\times g$ centrifugation for 10 min and at $360\times g$ for another 20 min, and stained with Wright–Giemsa dye, respectively. Briefly, Wright–Giemsa A solution was dropped and covered the whole specimen for 1 min, then B solution was added and fully mixed with A solution for another 30 min, followed by washing with water stream. Blood smears were observed by optical microscopy.

Platelet activation analysis

Platelets from four independent patients with lung cancer were resuspended with PBS and stained with antibodies including anti-CD62P–FITC or/and anti-CD41–APC or with a monoclonal immunoglobulin G (IgG) 1-FITC/IgG1–APC isotype control (all from Biolegend, San Diego, CA, USA) according to the manufacturer's protocol. After incubation for 30 min in the dark, a total of 20000 platelets events were acquired on a FACSAria II flow cytometer (BD, Biosciences, USA), and the data were analyzed using FlowJo Software 7.6 (Treestar, Inc., San Carlos, CA).

Platelet isolation and total RNA extraction

Platelet isolation was performed as described previously (Best et al. 2017). Briefly, peripheral blood was collected in EDTA-coated purple-capped Vacutainer tubes (BD, Franklin Lakes, NJ, USA) and stored at 4 °C until processing (within 6 h). To isolate platelets, platelet rich plasma (PRP) was separated from blood cells by twice centrifugations with $120\times g$ for 10 min, followed by another $360\times g$ centrifugation for 20 min to pellet platelets. Centrifugations were performed at room temperature. Total RNA was extracted from platelets using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and stored at − 80 °C according to the manufacturer's protocol.

mRNA microarray

Platelets were obtained from 2 ml peripheral blood of 4 healthy and 4 lung cancer patients and subjected for microarray. The Human RNA 4 × 180 K Expression Microarray (Agilent, Santa Clara, CA, USA) and the *LC Sciences* service (Hangzhou, Zhejiang, China) to process the samples were applied to genome-wide analysis. Briefly, a total of 2 μg RNA from each sample isolated with the RNeasy Mini kit (Qiagen, Hilden, Germany) was labeled with the Agilent Gene Expression Hybridization Kit (Agilent), after which, the slides were scanned with the Agilent Microarray Scanner (Agilent). A mixture of equal amounts of total RNAs from each group was used as the reference pool. The Feature Extraction software (version 10.7.1.1, Agilent) was used to analyze array images to get raw data and Genespring software (version 14.8, Agilent) was employed to finish the basic analysis with the raw data. R language was utilized for analyzing the differentially expressed mRNAs.

Quantitative PCR (qPCR)

The RNA was reversed transcribed into cDNA using the Takara PrimeScript RT reagent Kit (Takara Bio, Kusatsu, Japan) in 20 μl reaction. The expression of mRNAs was determined on LightCycler 480 qPCR system (Roche Diagnostics, Germany). The reactions were performed in a volume of 25 μl (12.5 μl of UltraSYBR Mixture (CWBio, Beijing, China), 0.5 μl of forward premier, 0.5 μl of reverse premier, 1 μl cDNA and 10.5 μl water). The reactions started at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min. The qPCR primers are listed in Table 1. All experiments were carried out in duplicate, and then the median Ct was calculated. *ACTB* was used as endogenous control. The relative expression was calculated using the comparative cycle threshold (ΔCt) method: ($\Delta Ct = Ct^{\text{target gene}} - Ct^{\text{ACTB}}$) as described previously (Ko et al. 2015).

Statistical analysis

The GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA) and SPSS 22.0 software (SPSS, Chicago, IL, USA) were used for statistical analysis. The Kolmogorov–Smirnov test was carried out to check the normality of the distribution. The normally distributed numeric variables were evaluated by parametric test, while non-normally distributed variables were analyzed by Mann–Whitney test; Kruskal–Wallis *H* test was used in comparisons among more than two groups. Receiving operating characteristic (ROC) curve analysis was used to evaluate the discriminatory power of the combinations. Data were shown as median ± interquartile range. P values < 0.05 were considered statistically significant and all tests were two-sided.

Results

Platelet quality control

To value the isolated platelet quality, platelets were stained with Wright–Giemsa staining. As shown in Fig. S1A, no white blood cells but a few red blood cells were observed after 120 × *g* centrifugation twice for 10 min, yet neither white blood cells nor red blood cells were detected under the microscope after 360 *g* for 20 min, consistent with the literature reports that a high-purity platelet preparation was determined by a ratio of < 5 karyocytes per 10^7 platelets (Best et al. 2015) (Fig. S1A). Then platelets from four independent patients with lung cancer were isolated and subjected to activation analysis, which demonstrated the activation level of platelets in lung cancer patients ranged 7.68–12.7%, as evidence from the CD62P$^+$/CD41$^+$ subpopulation (Fig. 1SB).

Identification of differentially expressed genes (DEGs) in platelet mRNA using microarray

To explore the DEGs, platelets were collected from four lung cancer patients and four healthy donors and subjected to mRNA microarray. A total of 688 up-regulated genes and 618 down-regulated genes in lung cancer patients were screened and drawn in a volcano map as shown in Fig. 1a, meanwhile the expression patterns of these DEGs were

Table 1 Primer sequences involved

Gene	Forward primer	Reverse primer
UQCRH	GAGGACGAGCAAAAGATGCTT	CGAGAGGAATCACGCTCATCA
MAX	CCGAGGTTTCAATCTGCGG	GAGGTCGTCAATATCTTGCTGG
MTURN	CGCAGGATGGATTTCTACGC	GCCCCAGTAAAGGTCTGAAAG
HLA-B	CAGTTCGTGAGGTTCGACAG	CAGCCGTACATGCTCTGGA
ACTB	CTGGAAGGTGGACAGCGAGG	TGACGTGGACATCCGCAAAG

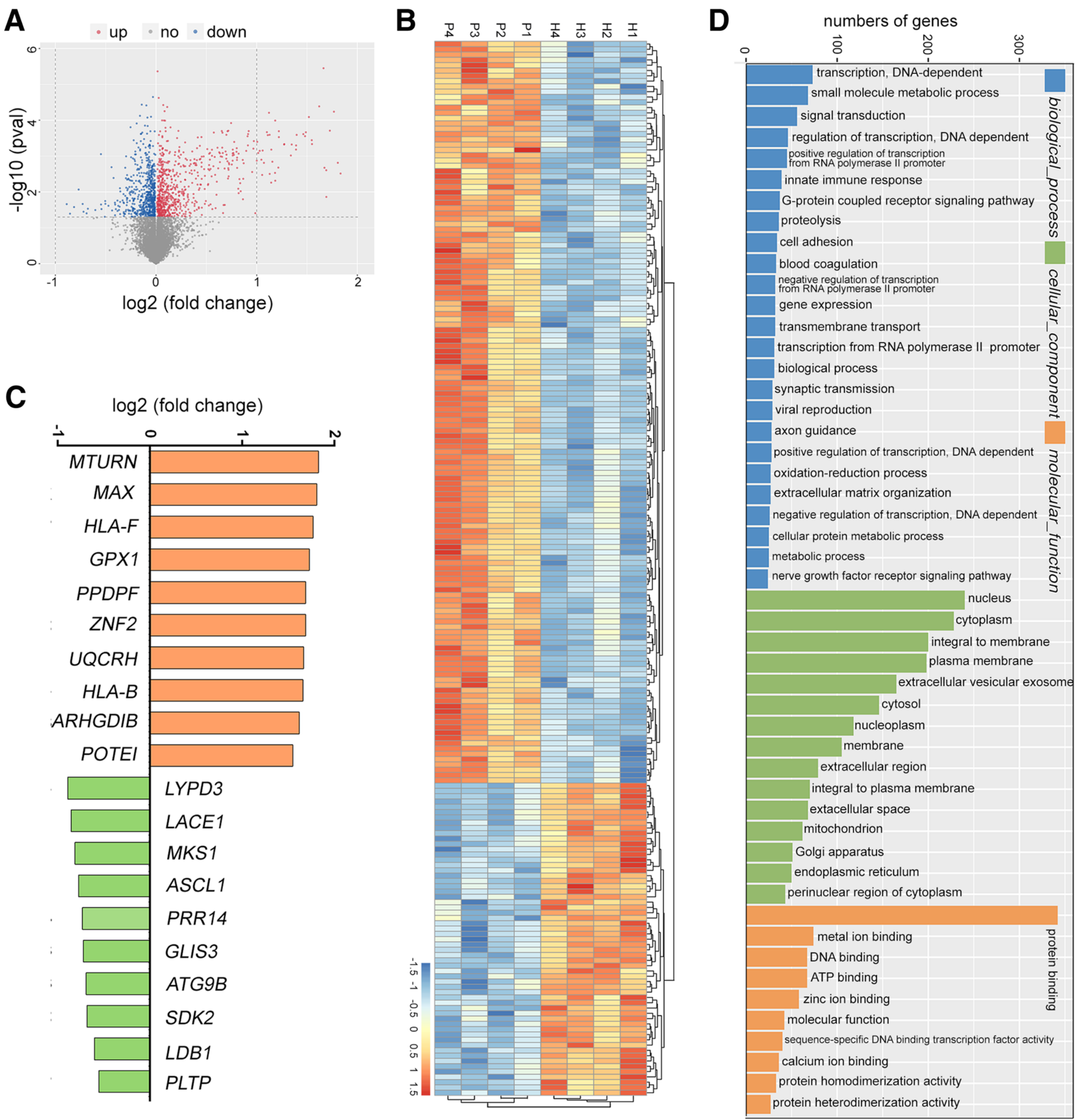

Fig. 1 Identification of platelet DEGs. **a** Volcano plot compared the expression fold change of platelet mRNA for lung cancer patients versus healthy donors; **b** a heat map was generated after supervised hierarchical cluster analysis. DEGs is shown in red (upregulation) versus blue (downregulation); **c** 20 selected DEGs are shown in yellow (upregulation) versus green (downregulation); **d** GO analysis of DEGs in lung cancer patients and healthy people

shown as the heatmap using hierarchical cluster analysis (Fig. 1b), and finally ten up-regulated and ten down-regulated with the most significance were selected from these DEGs as the candidates for next validation (as shown in Fig. 1c). Besides, the Gene Ontology (GO) functional classification was performed to gain overall insight into the functions of annotation genes. As shown in Fig. 1d, over 300 DEGs were involved in protein binding.

A three-platelet mRNA set: MAX, MTURN and HLA-B was elevated in lung cancer significantly

We then analyzed the 20 above selected platelet mRNAs expression by qPCR in a cohort with 48 lung cancer patients and 48 healthy donors. *UQCRH*, *MAX*, *MTURN*, *HLA-B* were identified and then subjected to a more expanded cohort for future validation. Detailed clinical characteristics of this cohort are provided in Table 2. The expression of *UQCRH*,

Table 2 Relationship between clinical characteristics and platelet *UQCRH, MAX, MTURN, HLA-B mRNA* expression

Characteristics	*UQCRH*		*MAX*		*MTURN*		*HLA-B*	
	No.	*P* value	No.	*P* value	No.	*P* value	No.	*P* value
Age								
> 64	93	0.3793	95	0.5710	70	0.6515	74	0.12
≤ 64	110		109		87		90	
Gender								
Male	137	0.1165	135	0.0584	106	0.004	113	0.7292
Female	66		69		51		51	
Smoking								
Yes	102	0.1880	106	0.5773	82	0.0239	88	0.2265
No	101		98		75		76	
Drinking								
Yes	62	0.8244	65	0.3695	54	0.0194	58	0.7297
No	141		139		103		106	
Histology								
AC	103	0.153*	100	0.083*	87	0.345*	82	0.810*
SCC	58		59		39		47	
SCLC	33		36		25		27	
Others	9		9		6		8	
Tumor length								
≤ 3 cm	78	0.5938	74	0.6936	58	0.4486	64	0.7975
> 3 cm	70		68		43		51	
Tumor metastasis								
Yes	70	0.5360	70	0.7211	52	0.3432	49	0.5930
No	133		134		105		115	

SCC squamous cell carcinoma, *AC* adenocarcinoma, *SCLC* small cell lung cancer

*Kruskal–Wallis *H* test

MAX, *HLA-B* in platelets of lung cancer was irrelevant with age, gender, smoking and drinking history, tumor size, pathological type and metastasis or not ($P > 0.05$), nevertheless the expression of *MTURN* was related to gender ($P = 0.004$), drinking ($P = 0.0194$) and smoking ($P = 0.0239$) (as shown in Fig. 2), but irrelevant with other clinical factors.

After Mann–Whitney tests analysis, the expression of platelet mRNA *UQCRH, MAX, MTURN* and *HLA-B* were confirmed which were elevated significantly in lung cancer compared with in healthy donors. As shown in Fig. 3a and Fig. S2A, in these four platelet mRNAs, *MAX*, *MTURN* and *HLA-B* were up-regulated in lung cancer with more significant differences ($P < 0.0001$, $P < 0.0001$ and $P = 0.0016$, respectively) than *UQCRH* ($P = 0.0286$). Therefore, we selected three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* as potential biomarker for lung cancer.

Next, we analyzed the three-platelet mRNA differential expression in early-stage lung cancer patients compared

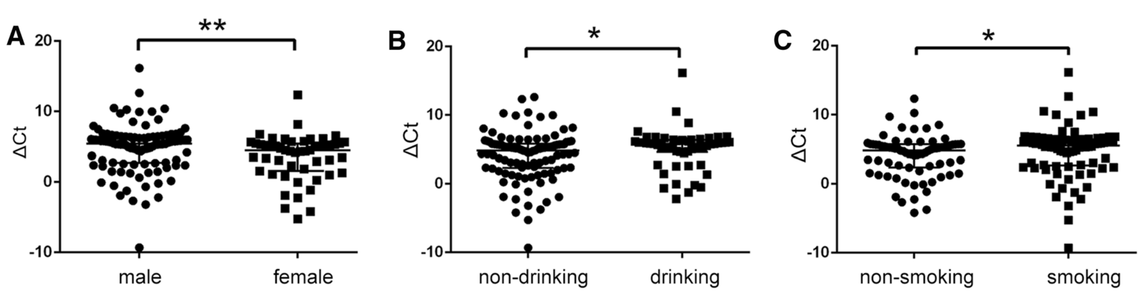

Fig. 2 The platelet *MTURN* mRNA was closely related with gender, smoking and drinking. The platelet *MTURN* mRNA demonstrated significant difference between male and female patients (**a**), between drinking and non-drinking patients (**b**), and between smoking and non-smoking patients (**c**). *$P < 0.05$; **$P < 0.005$

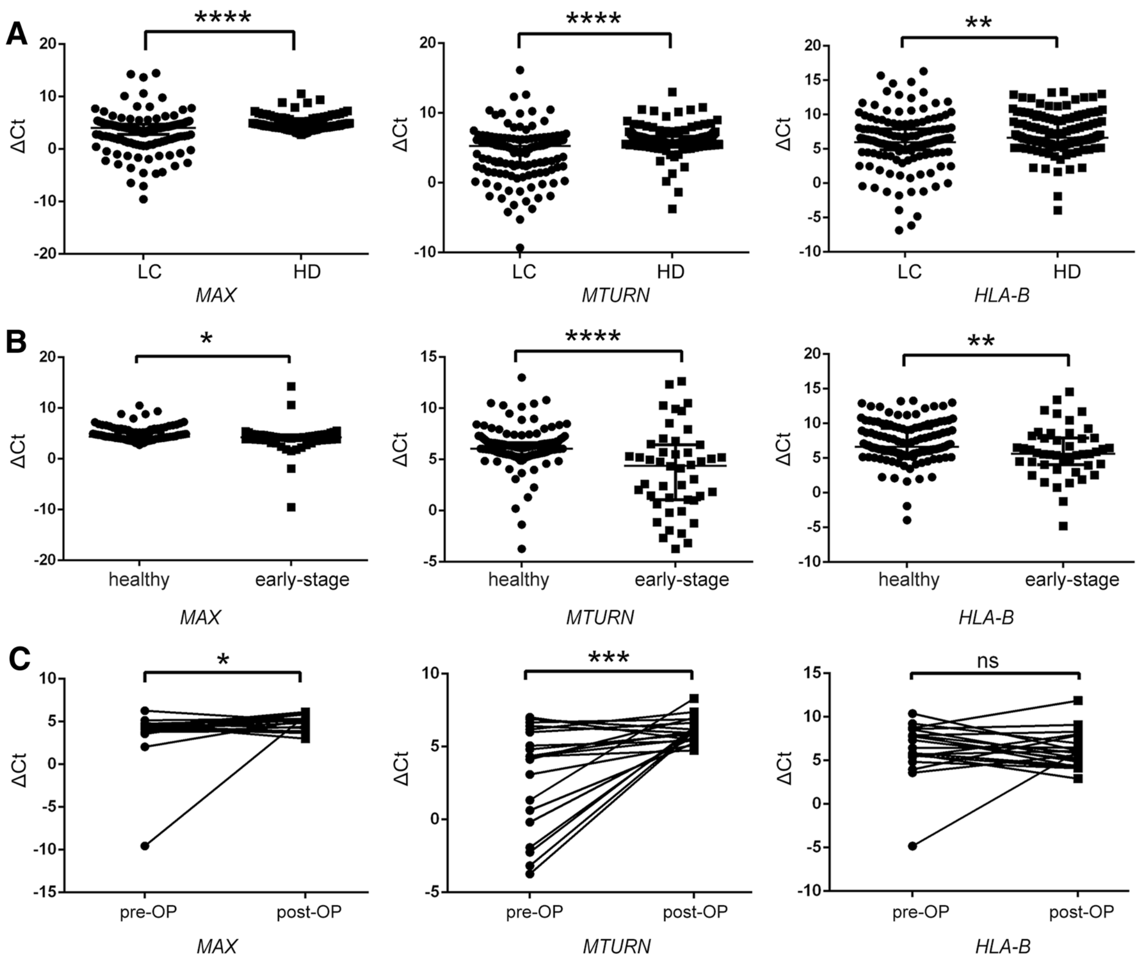

Fig. 3 A three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* was elevated in lung cancer significantly. The three genes of *MAX, MTURN, HLA-B* showed significant differences between the lung cancer patients and healthy donors (**a**), between the early-stage lung cancer patients and healthy donors (**b**), and between pre- and post-operation (**c**). *LC* patients with lung cancer, *HD* healthy donors, *pre-/post-OP* pre-/post-operation. *$P<0.05$; **$P<0.005$; ***$P<0.001$; ****$P<0.0001$, *ns* no significance

with that in healthy donors. Detailed clinical characteristics of this cohort are provided in Table 3 The expression of *MTURN*, *MAX*, *HLA-B* in platelets of early stage of lung cancer was irrelevant with age, gender, smoking and drinking history, tumor size and pathological type or not ($P>0.05$). As shown in Fig. 3b, The *MAX, MTURN* and *HLA-B* of platelet mRNA expressed higher in early stage lung cancer compared with those in healthy donors ($P=0.0115$, $P<0.0001$ and $P=0.007$).

In order to verify the relationship of *MAX*, *MTURN*, *HLA-B* and lung cancer, we analyzed the differences of these three-mRNA expression pre- and post-operation of lung cancer. The average time interval between surgical and post-operative samples was about 5 days (as shown in Table S1). As shown in Fig. 3c, *MAX* and *MTURN* were decreased after surgery significantly, indicating they were closely correlated with tumor occupying. However, *HLA-B* did not differ between pre- and post-operation, indicating it was irrelated with tumor occupying yet might be involved in tumor function.

A three-platelet mRNA set: MAX, MTURN and HLA-B as diagnostics biomarker for lung cancer

To evaluate diagnostic performance of the three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* for lung cancer, a receiver-operating characteristic (ROC) curve was calculated via comparing the 127 lung cancer samples with 82 healthy samples. As shown in Fig. 4a, the area under the curve (AUC) was 0.734 with a sensitivity of 60.6%, and a specificity of 81.7%. These results indicated that the three-platelet mRNA set might be a novel biomarker for lung cancer.

Similarly, ROC analysis of *MAX, MTURN* and *HLA-B* in 33 patients with early-stage lung cancer and 82 normal controls was used to determine cut-off values. As shown in Fig. 4b, the AUC was 0.787 with a sensitivity of 72.7%,

Table 3 Relationship between early clinical characteristics and *MAX, MTURN, HLA-B mRNA* expression

Characteristics	*MAX (54)*		*MTURN (46)*		*HLA-B (47)*	
	No.	*P* value	No.	*P* value	No.	*P* value
Age						
> 61/62/62	26	0.2955	21	0.7696	20	0.2119
≤ 61/62/62	28		25		27	
Gender						
Male	38	0.8179	33	0.2778	34	0.8046
Female	16		13		13	
Smoking						
Yes	27	0.3040	20	0.8342	23	0.4106
No	27		26		24	
Drinking						
Yes	19	0.6055	13	0.8412	18	0.5534
No	35		33		29	
Histology						
AC	36	0.754*	35	0.563*	33	0.469*
SCC	13		8		10	
SCLC	2		1		2	
Others	3		2		2	
Tumor length						
> 2 cm	19	0.56	13	0.1335	17	0.2074
≤ 2 cm	21		19		19	

SCC squamous cell carcinoma, *AC* adenocarcinoma, *SCLC* small cell lung cancer

*Kruskal–Wallis *H* test

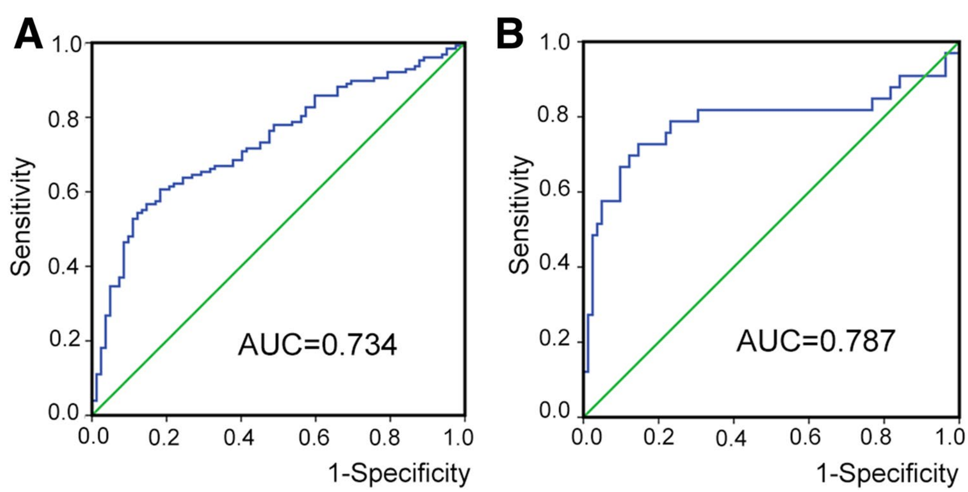

Fig. 4 A three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* as diagnostics biomarker for lung cancer. The ROC curve analysis of combination of three-platelet mRNA set: *MAX, MTURN* and *HLA-B* for lung cancer (AUC = 0.734) (**a**), and for early-stage lung cancer (AUC = 0.787) (**b**). AUC, area under curve

and a specificity of 85.4%, respectively, suggesting the potential diagnostic role of the three genes for early-stage lung cancer. These results indicate that the three-platelet mRNA set levels might be used for early detection of tumors.

Notably, as shown in Fig. 5a, platelet *MTURN* mRNA expressed higher in female patients with lung cancer patients than that in female healthy donors, which possessed a dramatically high diagnostic efficiency, its AUC for women was 0.825 with a sensitivity of 84.7%, and a specificity of 72.5%. In this female patient cohort, never-smokers predominated over 92%, adenocarcinoma predominated over 71%. Although platelet *MTURN* mRNA were also up-regulated in male patients with lung cancer, its diagnostic efficiency seemed much lower, the AUC was 0.637 with a sensitivity of 80.6%, and a specificity of 50.9% (Fig. 5b).

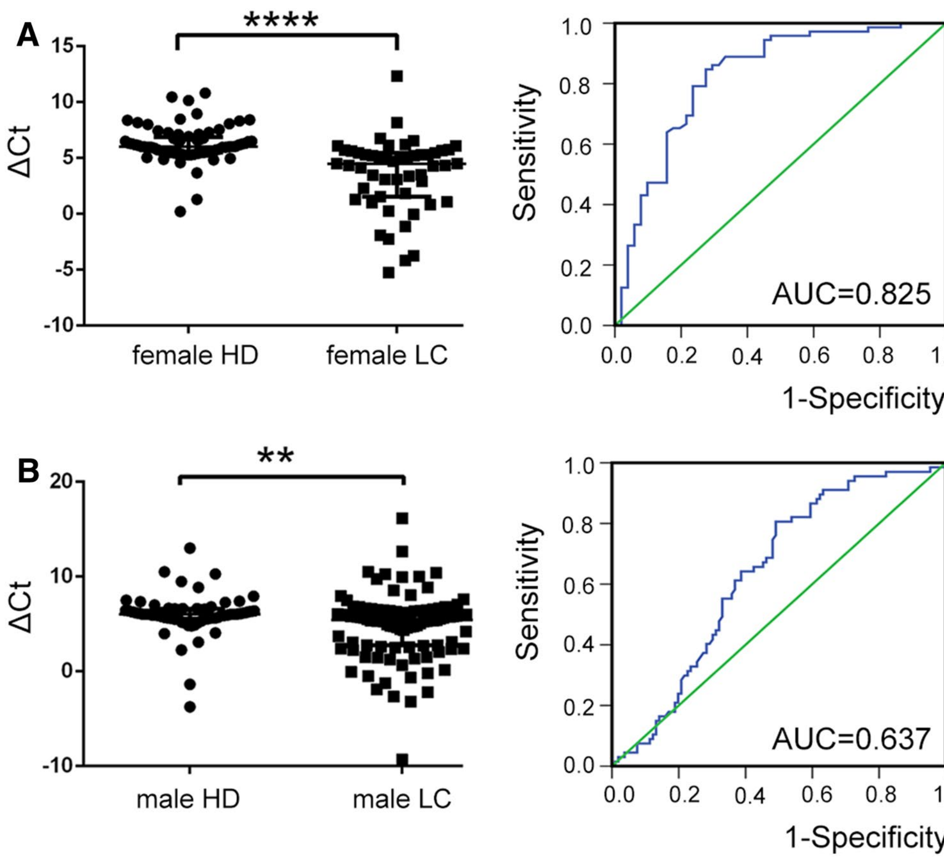

Fig. 5 The platelet *MTURN mRNA* as diagnostics biomarker for female lung cancer. Platelet *MTURN* mRNA expressed higher in LC than that in HD (left), a ROC curve of platelet *MTURN* mRNA was drawn for lung cancer (right) in female (**a**), or in male (**b**). *LC* patients with lung cancer, *HD* healthy donors, *AUC* area under curve. $^{**}P<0.005$; $^{****}P<0.0001$

A three-platelet mRNA set: *MAX, MTURN* and *HLA-B* for chemotherapy response assessment

Next, we researched the three-platelet mRNA levels for chemotherapy response assessment. In our study, 40 patients with lung cancer received first-line chemotherapy. Detailed clinical characteristics of this cohort were provided in Table S2. Response rates (RR) was defined as the proportion of response evaluable patients with complete response (CR) and partial response (PR) as best response; disease control rates (DCR) was defined as the proportion of response evaluable patients with CR, PR and stable disease (SD) as best response (Finn et al. 2011). RR to the first-line chemotherapy in the *MAX*, *MTURN* and *HLA-B* were 63.9%, 68%, 69.6%, respectively. In addition, DCR with the first-line chemotherapy in the *MAX, MTURN and HLA-B* groups were 91.7%, 96%, 95.7% (as shown in Table 4).

Besides, we divided these patients into PR and non-PR groups. Non-PR included progressive disease (PD) and stable disease (SD). No patients had a CR in our cohort. The *MAX* had 23 patients of PR, 13 of non-PR; The *MTURN* had 17 patients of PR, 8 of non-PR and *HLA-B* had 16 patients of PR, 7 of non-PR. We found that these genes are associated with chemotherapeutic effect, mRNA expression of this three-platelet was correlated with "favorable" first chemotherapy response ($P=0.0332$, $P=0.0482$, $P=0.0266$, respectively) (Fig. 6), thus indicating *MAX, MTURN, HLA-B* might act as a potential biomarker to predict chemotherapeutic effect. In addition, the *UQCRH* had similar characteristics to the above three genes (as shown in Table S2, S3 and Fig. S2B).

Table 4 Response to the first chemotherapy in the three-platelet mRNA set: MAX, MTURN and HLA-B cohort

Gene	*N*	CR	PR	SD	PD	RR%	DCR%
MAX	36	0	23	10	3	63.9	91.7
MTURN	25	0	17	7	1	68	96
HLA-B	23	0	16	6	1	69.6	95.7

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *RR* response rate, *DCR* disease control rate

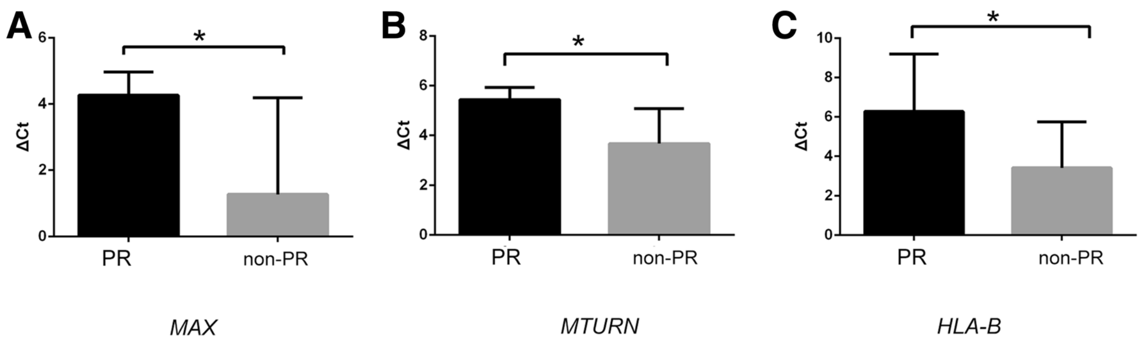

Fig. 6 A three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* for chemotherapy response assessment. Low mRNA expression of *MAX* (**a**), *MTURN* (**b**), *HLA-B* (**c**) was correlated with favorable first chemotherapy response. *PR* partial response

Discussion

The morbidity and mortality of lung cancer are gradually increasing and most patients are diagnosed at an advanced stage of lung cancer (Fervers 2011). Thus, sensitive and specific biomarkers to identify lung cancer patients are urgently needed. The clinical application of liquid biopsy in lung cancer progressively has proved a pivotal tool for screening and early detection of cancer, such as TEPs (Best et al. 2018), which have emerged as key players in tumor cell growth, establishment of distant metastasis (Goubran et al. 2014). In our study, we screened platelet DEGs and validated their diagnosis values for lung cancer, suggesting that platelets had unique characteristics that enabled them a potential novel tool for diagnostics.

In current study, *MAX*, *MTURN* and *HLA-B* expression levels were statistically increased in the platelets from lung cancer patients compared to those from healthy controls, possessing rather high diagnostic efficiency, the AUC of three-platelet mRNA set was 0.734 with a sensitivity of 60.6%, and a specificity of 81.7%. More importantly, they significantly expressed higher in early-stage lung cancer than in healthy donors, which also possessed high diagnostic efficiency with AUC of 0.787, a sensitivity of 72.7%, and a specificity of 85.4%, suggesting a three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* acts as the novel biomarker for lung cancer diagnostics especially early diagnostics.

MAX (MYC associated factor X), a member of the basic helix–loop–helix leucine zipper (bHLHZ) family of transcription factors, encodes a ubiquitously expressed phosphoprotein with two commonly expressed isoforms that migrates as 21 and 22 kDa proteins. Together with its heterodimerization partner c-Myc, it forms a transcription factor complex and mediates its tumor-promoting properties through the induction of growth control genes (Boxer and Dang 2001; Schmidt 1999), and participates in cell proliferation, differentiation and apoptosis (Blackwood et al. 1992). High levels of *MAX* may enhance stress-induced FasL expression in lung cancer cells, thereby protecting cancer cells from damage to the immune system (Bennett et al. 1998; Wiener et al. 2004). In our study, we found that the expression of platelet *MAX* mRNA in the lung cancer group or early-stage lung cancer was higher than that in the normal group, indicating that *MAX* might take part in the tumor progression.

MTURN (maturin, neural progenitor differentiation regulator homolog) is conservative in evolution and has little information about its expression, function or protein structure. It encodes an acidic, evolutionarily conserved protein, which is essential for normal primary neurogenesis (Martinez-De Luna et al. 2013), differentially expressed during human embryonic development (Yi et al. 2010). It is reported that *MTURN* participates in MAPK/ERK, SAPK/JNK and NF-κB signaling pathway (Sun et al. 2014), indicating the potential role in tumorigenesis. Our data reported higher platelet *MTURN* expression in lung cancer. Accumulating evidence have demonstrated that lung cancer in never-smokers is clinically characterized by a higher occurrence of adenocarcinoma and an increased incidence in female widely particularly in Asia (Lan et al. 2012), one-third lung cancer cases arise in individuals who never smoked (Zhou and Zhou 2018). Coincidently, never-smokers predominated over 92%, adenocarcinoma predominated over 71% in our female patient cohort. Notably, the platelets *MTURN* mRNA was closely associated with gender and smoking history ($P=0.004$ and $P=0.0239$), which was higher in female patients than that in female healthy donors, possessing dramatically high diagnostic efficiency, its AUC for women was 0.825 with a sensitivity of 84.7%, and a specificity of 72.5%, thus providing a novel and reliable diagnostics biomarker for non-smoking female lung cancer.

HLA-B (human leukocyte antigen, class I, B) belongs to the HLA class I heavy chain paralogues. This class I molecule is a heterodimer consisting of a heavy chain and a light chain (beta-2 microglobulin). It expresses in nearly all cells, plays key roles in the immune response by presenting endogenous antigens to CD8-positive T cells (van der Bruggen et al. 2007), whose abnormal expression enables tumor cells to hide tumor-derived antigens and consequently evades recognition and killing capacity by CTLs (Sette et al. 2001). Besides, *HLA-B* may play an important role in p300-mediated p53 acetylation in a variety of cancers (Sasaki et al. 2007), such as colon cancer (Goodman and Smolik 2000). and also in apoptotic cell death induced by papillomavirus binding factor in osteosarcoma (Tsukahara et al. 2009). Moreover, *HLA-B* was elevated in the platelet of lung cancer which contrasted with the low expression in many cancer samples (Nestle et al. 1998) such as thyroid cancer (Haghpanah et al. 2009) and breast cancer (Cantu de Leon et al. 2009). This phenomenon might be explained by that the up-regulated *HLA-B* might originate from tumor microenvironment rather than from tumor itself, since in current research *HLA-B* expression did not differ between pre- and post-operation, as well as was increased in leukocytes of lung cancer patients compared with that of healthy donors by RNA-seq (data not shown), indicating it was irrelated with tumor occupying and involved in tumor function. Conversely, *MAX* and *MTURN* were significantly decreased after surgery, indicating they were closely correlated with tumor occupying.

In this study, we evaluated the association between platelet *MAX*, *MTURN* and *HLA-B* mRNA expression and first chemotherapy response, respectively. Our findings indicated that low mRNA expression of this three-platelet was correlated with "favorable" first chemotherapy response, thus providing a noninvasive marker to predict first chemotherapy response.

Several limitations should be carefully considered in the present study. First, our results included 225 patients with lung cancer, the total sample sizes were small and might lack statistically vigorous power, only 40 patients had the detailed information of chemotherapy. Second, platelet *MTURN* mRNA had increased expression in female patients with lung cancer and processed a promising diagnostic efficiency. Although in our cohort 92% females were never-smokers, we failed to analyzed role of platelet *MTURN* mRNA in the sub-cohort of lung cancer in never-smokers (LCINS) due to lack of smoking history information in healthy donors.

In summary, our results demonstrated that a three-platelet mRNA set: *MAX*, *MTURN* and *HLA-B* was up-regulated in lung cancer patients compared to in healthy donors, and valuable for diagnostics of lung cancer especially for early diagnostics, in which platelet *MTURN* mRNA processed a dramatically high diagnostic efficiency in female patients with lung cancer. More importantly, low mRNA expression of this three-platelet was correlated with "favorable" first chemotherapy response, thus providing the evidence this three-platelet mRNA set as a biomarker for lung cancer.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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