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Effects of polyunsaturated fatty acids on gastric cancer immunity and immunotherapy

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Although immunotherapy has been predominant for advanced gastric cancer treatment, it still has limitations. Polyunsaturated fatty acids (PUFAs) are associated with inflammation while their roles in tumor immune microenvironment (TIME) are unclear. This study explores PUFAs' impacts on gastric cancer immunity and immunotherapy efficacy. Bioinformatics analysis was conducted to identify differential expressions of PUFAs metabolic genes and their immune correlations. Clinical data of advanced gastric cancer patients receiving immunotherapy at Zhongda Hospital (2020–2024), whose serum PUFAs were measured by mass spectrometry (MS), were collected and analyzed. Bioinformatics analysis revealed differential expression of half of PUFAs metabolic genes in gastric cancer. PUFAs metabolism towards Omega-3 (ω-3) tended to increase infiltration of active anti-tumor cells and up-regulate multiple immune checkpoints expressions, while metabolism towards Omega-6 (ω-6) led to opposite TIME outcomes. The clinical study demonstrated that lower serum ω-6/ω-3 ratio, arachidonic acid/eicosapentaenoic acid (AA/EPA) ratio, linoleic acid/alpha-linolenic acid (LA/ALA) ratio and higher EPA were associated with better six-month progression-free survival rate (6-month PFS) and one-year overall survival rate (1-year OS). This study deepens our understandings of TIME in gastric cancer. It clearly demonstrates that maintaining appropriate PUFAs ratios or values is promising in improving prognosis.

Keywords Gastric cancer, PUFAs, Tumor immune microenvironment, Immunotherapy, Bioinformatics analysis, Mass spectrometry

Gastric cancer is a highly lethal malignancy and is among the leading causes of cancer-related deaths globally. During the past decade, the combination of immune checkpoints blockers (ICBs) and chemotherapy has been dominant in the first-line treatment of advanced gastric cancer[1,2]. ICBs target co-inhibitory molecules such as PD-1/PD-L1 and CTLA-4 to relieve immune suppression in tumor-infiltrating immune cells, thereby exerting their anti-tumor effects[3]. However, immunotherapy has limitations in predictive biomarkers, as its efficacy is not always consistent with commonly used PD-L1 expression, mismatch repair proteins (MMR) status, microsatellite instability (MSI) status, and tumor mutation burden (TMB)[4,5]. Moreover, the current unsatisfactory immunotherapy efficacy, with a median overall survival ranging from 11 to 14 months and median progression-free survival around 6 months[6], indicates the need for further exploration of the tumor immune microenvironment (TIME) in gastric cancer.

Polyunsaturated fatty acids (PUFAs) are fatty acids containing two or more unsaturated bonds. Based on the position of the first unsaturated bond at the methyl end, they can be categorized into several types: Omega-3 (ω-3), Omega-6 (ω-6), Omega-7 (ω-7), and Omega-9 (ω-9). ω-3 and ω-6 polyunsaturated fatty acids are closely associated with inflammatory reactions, despite their low blood levels and non-participation in cellular energy supply. The ω-3 family primarily includes eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and alpha-linolenic acid (ALA); the ω-6 family mainly comprises arachidonic acid (AA), linoleic acid (LA), dihomo-gamma-linolenic Acid (DGLA) and gamma-linolenic acid (GLA). Most of these PUFAs can only be obtained through diet or by conversion from other PUFAs[7,8]. Their downstream metabolites are catalyzed in strict order by enzyme systems from different families, including phospholipase A2 (PLA2)[9,10], fatty acids desaturase (FADS)[11], elongase enzymes (ELOLV)[12], cyclooxygenase (COX) and lipoxygenase (LOX)[13]. PUFAs serve as potent chemotactic factors and inflammation modulators, playing key roles in the balance between the initiation and resolution of inflammation[14]. These processes are mediated through two main mechanisms, which are ligand-receptor recognition[15,16] and alterations of cell

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membrane compositions[14,17]. Aberrations in PUFAs metabolism can provoke intractable chronic inflammation, thereby increasing the risk of carcinogenesis[18]. (Fig. 1) Recent studies found out that the relationship between PUFAs patterns and tumor immunity is far more complex than initially imagined. Their various components are closely linked to tumor cell apoptosis and ferroptosis, the antigen presentation ability of tumor-infiltrating immune cells, the intensity of cytotoxic effects, and even resistance to chemotherapy and immune checkpoint inhibitors[19–24]. From another angle, gastrointestinal mucosa develops a distinctive TIME in different aspects as a unique immune barrier and digestive organ[25], with prominent clustering of tumor-associated macrophages (TAMs), regulatory T cells (Tregs) and aberrant natural killer (NK) cells[26–28].

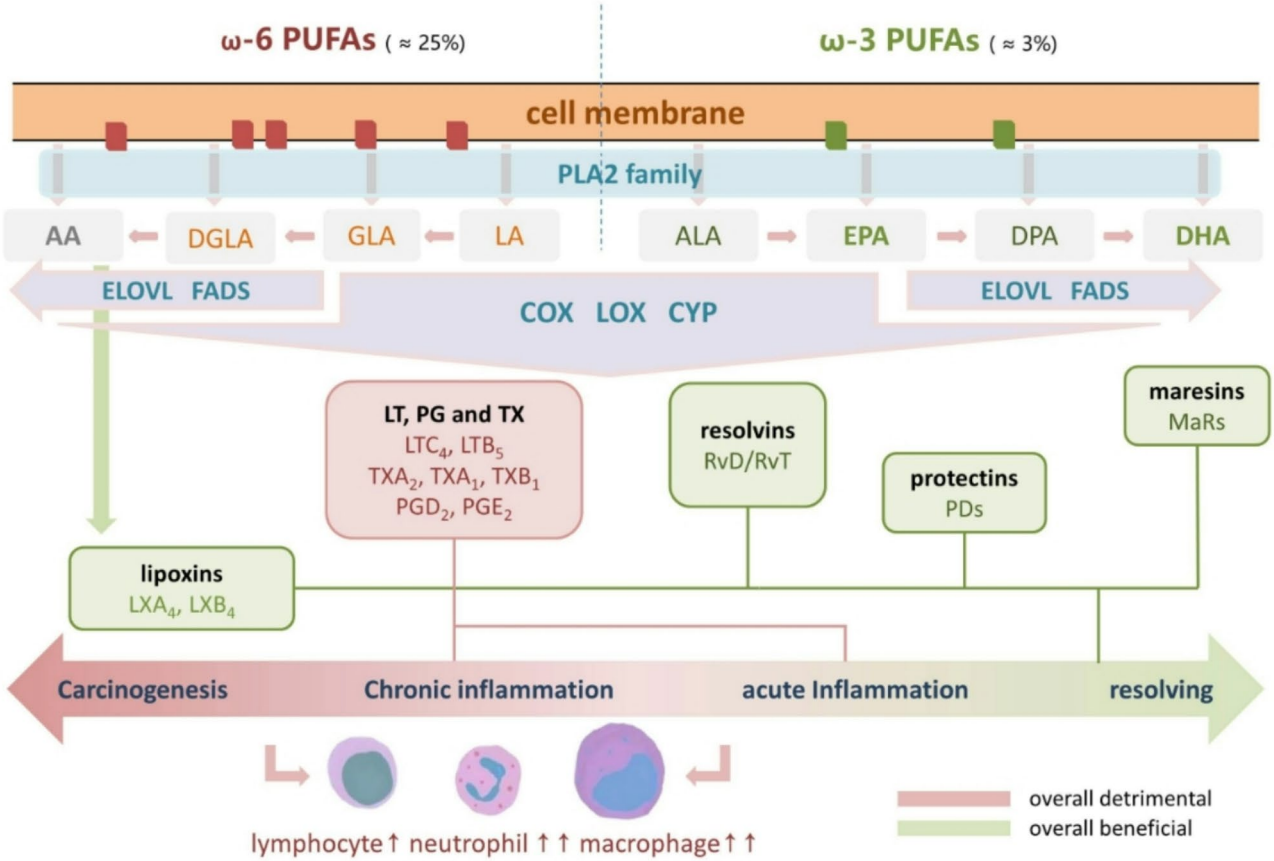
Hitherto, either basic studies or clinical studies on PUFAs have been sporadic and relatively independent. In the meantime, it remains the most urgent challenge to fully utilize immune reactions against tumor cells. Based on previous studies, we believe that PUFAs might be significant in shaping the immune landscape in gastric TIME. Our study integrated bioinformatics analysis and clinical analysis to explore the influence of PUFAs on the gastric TIME and the efficacy of immunotherapy.

Materials and methods

Bioinformatics analysis

Data obtainment, quality control and pre-processing

Datasets were downloaded from public database Gene Expression Omnibus (GEO. https://www.ncbi.nlm.nih.gov/geo/). GSE118916 and GSE13911 (Expression profiling by array) were picked for their better quality and wider range of citations[29–33]. Datasets quality control and pre-processing were completed by RStudio 4.4.1.



PUFAs: polyunsaturated fatty acids; **LA**: linoleic acid; **ALA**: alpha-linolenic acid; **GLA**: gamma-linolenic acid; **DGLA**: dihomo-gamma-linolenic Acid; **AA**: arachidonic acid; **EPA**: eicosapentaenoic acid; **DPA**: docosapentaenoic acid; **DHA**: docosahexaenoic acid; **LT**: leukotriene; **PG**: prostaglandin; **TX**: thromboxane; **RvD/RvT**: resovinD/resolvinT; **PDs**: protectins; **MaRs**: maresins.
COX: cyclooxygenase; **LOX**: lipoxygenase; **CYP**: cytochrome P450; **ELOVL**: elongation of very long chain fatty acids protein; **FADS**: fatty acid desaturase; **PLA2**: phospholipase A2.

Fig. 1. The network of polyunsaturated fatty acids. It presents main PUFAs' downstream metabolites and their inflammation correlations. ω-6 PUFAs metabolites like leukotrienes (LT), prostaglandins (PG) and thromboxanes (TX) generally lead to chronic inflammation and carcinogenesis, while ω-3 PUFAs produce resolvins, protectins and maresins which are involved in resolving acute inflammation.

Differentially expressed genes (DEGs) and enrichment analysis

Data preprocessing and differentially expressed genes analysis were performed using online analyzing tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/). Genetic Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)[34] enrichment analysis were performed based on DAVID database (http://david-d.ncifcrf.gov/).

Tumor microenvironment immune infiltration

Immune infiltration analysis was performed using CIBERSORT method on RStudio 4.4.1. PPI network visualization was completed by cytoscape based on STRING database (http://string-db.org/). "PUFAs metabolism score" was designed in this section to evaluate metabolic depth towards different directions. It refers to sum of expression levels (based on raw expression matrix) of genes coding main PUFAs metabolism related enzymes. ω-6 PUFAs metabolism score was calculated by expression levels of PLA2G4A, PLA2G6, PTGS1, PTGS2, ALOX5 and ALOX5A (defined as Gene Set A). ω-3 PUFAs metabolism score was calculated by expression levels of FADS2, ELOVL2, ALOX5AP and ALOX15 (defined as Gene Set B). Calculations are presented as follows:

$$\omega-6\ \text{PUFAs metabolism score} = \sum(\text{Gene Set A})$$

$$\omega-3\ \text{PUFAs metabolism score} = \sum(\text{Gene Set B})$$

$$\omega-6/\omega-3\ \text{PUFAs metabolism ratio score} = \frac{\sum(\text{Gene Set A})}{\sum(\text{Gene Set A})+\sum(\text{Gene Set B})}$$

Considering the possible bias rendered by joint analysis for arrays' discrepancy in expression scale, only GSE13911 was included.

Serum PUFAs determined by mass spectrometry

Mass spectrometry (MS) was utilized as a precise analyzing tool in our study. Patients' blood samples were centrifuged promptly after collection, and were stored at −80 °C. When running analysis, stored serum was then extracted and preprocessed properly before being injected into the gas chromatograph. Eluted fatty acid derivatives then entered the mass spectrometer. Electron ionization was applied, and the ions were accelerated into a mass analyzer and separated based on mass-to-charge ratio, thus forming mass spectra. Peak intensities of specific fatty acid derivatives were measured and compared with calibrated standards to determine fatty acid concentrations. The mass spectrometer uses an electron impact ionization source (EI) with an ionization energy set at 70 eV, a scanning range of m/z 50–500, a scanning speed of 10 times per second, an ion source temperature maintained at 230 °C, and an interface temperature of 280 °C. Data from the mass spectrometer were processed and organized on the computer software XCMS. Molecular formula and characteristic peaks of measured PUFAs are presented as follows (Table 1). It should be declared that all patients' blood samples were collected for multiple clinical diagnosis and treatment purposes with the consent of Zhongda Hospital, Southeast University in accordance with relevant guidelines and regulations.

Statistical analysis of clinical data

Clinical data of patients who received immunotherapy from 2020 to 2024 at Zhongda Hospital, Southeast University were collected. All Included patients had advanced or recurrent gastric cancer and received ICBs monotherapy or combination therapy. Their serum PUFAs levels were determined by mass spectrometry at the beginning of treatment. They received regular treatment and follow-up with complete clinical data for

Name	Molecular formula	Structural formula	Mass spectrometry characteristic peaks (m/z)[a]
C16:1	$C_{16}H_{30}O_2$	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	**254** (M^+), **236** ($M{-}H_2O$), **211** ($M{-}CH_3CH_2$), **129** ($CH_3(CH_2)_5CH{=}CH$)
C18:1 (OA)	$C_{18}H_{34}O_2$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	**282** (M^+), **264** ($M{-}H_2O$), **239** ($M{-}CH_3CH_2$), **157** ($CH_3(CH_2)_7CH{=}CH$)
γ-C18:3 (GLA)	$C_{18}H_{30}O_2$	$CH_3(CH_2)_4(CH{=}CHCH_2)_3(CH_2)_4COOH$	**278** (M^+), **260** ($M{-}H_2O$), **235** ($M{-}CH_3CH_2$), **179** ($CH_3(CH_2)_4(CH{=}CHCH_2)_2$), **135** (CH3(CH2)3CH=CHCH2)
α-C18:3 (ALA)	$C_{18}H_{30}O_2$	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	**278** (M^+), **260** ($M{-}H_2O$), **235** ($M{-}CH_3CH_2$), **179** ($CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2$), **151** (CH3CH2CH=CHCH2CH=CHCH2)
C18:2 (LA)	$C_{18}H_{32}O_2$	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	**280** (M^+), **262** ($M{-}H_2O$), **237** ($M{-}CH_3CH_2$), **155** ($CH_3(CH_2)_4CH{=}CHCH_2$)
ω-6-C22:5	$C_{22}H_{34}O_2$	$CH_3(CH_2)_4(CH{=}CHCH_2)_5(CH_2)_2COOH$	**334** (M^+), **316** ($M{-}H_2O$), **291** ($M{-}CH_3CH_2$), **219** ($CH_3(CH_2)4(CH{=}CHCH_2)_3$), **127** (CH3(CH2)4CH=CHCH2)
ω-3-C22:5 (DPA)	$C_{22}H_{34}O_2$	$CH_3CH_2(CH{=}CHCH_2)_5(CH_2)_2COOH$	**334** (M^+), **316** ($M{-}H_2O$), **291** ($M{-}CH_3CH_2$), **219** ($CH_3CH_2(CH{=}CHCH_2)_3$), **143** (CH3CH2CH=CHCH2CH=CHCH2)
C20:5 (EPA)	$C_{20}H_{30}O_2$	$CH_3CH_2(CH{=}CHCH_2)_5(CH2)_2COOH$	**302** (M^+), **284** ($M{-}H_2O$), **259** ($M{-}CH_3CH_2$), **191** ($CH_3CH_2(CH{=}CHCH_2)_3$)
C22:6 (DHA)	$C_{22}H_{32}O_2$	$CH_3CH_2(CH{=}CHCH_2)_6CH2COOH$	**328** (M^+), **310** ($M{-}H_2O$), **285** ($M{-}CH_3CH_2$), **213** ($CH_3CH_2(CH{=}CHCH_2)_4$)
C20:4 (AA)	$C_{20}H_{32}O_2$	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	**304** (M^+), **286** ($M{-}H_2O$), **261** ($M{-}CH_3CH_2$), **183** ($CH_3(CH_2)_4(CH{=}CHCH_2)_2$)

Table 1. Molecular formula and characteristic peaks of measured PUFAs. [a]MS param.: EI, 70 eV; scan range m/z 50–500; scan speed 10/s; ion source temp. 230 °C; interface temp. 280 °C.

assessment. Patients meeting the following conditions were excluded: those with recent severe infections, injuries, major surgeries, etc. that could cause inflammatory reactions and affect blood test results; those who had recently fasted or been deprived of water, or used enteral or parenteral nutrition that could interfere with blood test results; those whose initial ECOG scores (physical condition score designed by Eastern Cooperative Oncology Group) exceeded 1 point.

Clinical data included age, sex, stage, ECOG score, history of radiotherapy, treatment line, history of smoking, immunotherapy indications (dMMR/MSI-H/PD-L1 CPS>1/PD-L1 TPS>1%/TMB>10), immunotherapy regimens and determined PUFAs values. Evaluation indicators for the efficacy of immunotherapy included objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), six-month progression-free survival rate (6-month PFS) and one-year overall survival rate (1-year OS). Their definitions stick to the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST1.1). Data analysis was completed using SPSS v26.0 and RStudio 4.4.1.

Results

Differentially expressed genes (DEGs) and enrichment analysis

Parallel comparison of GSE13911 and GSE118916 revealed overall consistent DEGs related to PUFAs metabolism between gastric tumor tissues and adjacent normal tissues (Fig. 2a,b). The absolute value of the log2 fold change ($|\log FC| \geq 0.5$, where it indicates that the up- or down-regulation degree exceeds 1.4 times) and $P < 0.05$ were set as the analytical thresholds for DEGs analysis. It manifested that half of known PUFAs related genes were differentially expressed, which enriched mostly in catalyzing ω-6 PUFAs metabolism (highlighted with red in Fig. 2b). These genes mainly included FADS1, PLA2G2A, PLA2G4A, PTGS2 and ALOX5. ω-3 PUFAs metabolism related genes (highlighted with green in Fig. 2b) including ELOVL5, ELOVL2, CYP4F2, ALOX15, CYP2J2, CYP2U1, FADS2 showed insignificant expression difference or slight down-regulation. Most of shared enzymes of ω-3 and ω-6 PUFAs from PLA2, ELOVL and CYP families (highlighted with black in Fig. 2b) were mostly down-regulated. Genetic ontology (GO) enrichment analysis manifested varying degrees of alterations in lipid metabolism (Fig. 2c). When it comes to PUFAs, these biological processes could cover extension of fatty acids carbon chains, desaturation and translocation of double bonds, absorption and digestion and membrane-crossing transport. Combination of DEGs and GO enrichment results manifested crosstalk between PUFAs, membrane signaling and inflammation (Fig. 2e). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis revealed that main alterations in PUFAs metabolism enrich in linoleic acid metabolism and arachidonic acid metabolism of ω-6 family (Fig. 2d).

PUFAs and gastric cancer microenvironment (TME) immune cell infiltration

Analysis of 69 samples of GSE13911 including tumor and normal tissues was completed using CIBERSORT method. Results showed that six categories of immune cell, which were plasma cells ($P < 0.001$), activated $CD4^+$ memory T cells ($P < 0.001$), follicular Th cells ($P = 0.016$), M0 macrophages ($P < 0.001$), M1 macrophages ($P < 0.001$), M2 macrophages ($P = 0.005$), resting mast cells ($P = 0.005$) and neutrophils ($P = 0.003$) had significant infiltration differences between normal gastric tissue and tumor tissue.

Further analysis based on our experimental tool "PUFAs metabolism score" showed that higher ω-3 PUFAs metabolism score was linked with higher infiltration of $CD4^+$ memory T cells ($P = 0.019$), follicular Th cells ($P = 0.023$), γδ T cells ($P = 0.011$), M1 macrophages ($P = 0.048$), M2 macrophages ($P = 0.039$) and neutrophils ($P = 0.012$). And higher ω-3 PUFAs metabolism score was also linked with lower infiltration of plasma cells ($P < 0.001$), resting NK cells ($P = 0.017$) and regulatory T cells ($P = 0.010$) (Fig. 3a). Higher ω-6 PUFAs metabolism score was linked with higher infiltration of neutrophils ($P = 0.018$) and lower infiltration of resting $CD4^+$ memory T cells ($P = 0.013$). Higher ω-6/ω-3 PUFAs metabolism score was linked with higher infiltration of plasma cells ($P < 0.001$), regulatory T cells ($P = 0.033$), resting NK cells ($P = 0.015$) and activated dendritic cells ($P = 0.008$) and lower infiltration of γδ T cells ($P = 0.017$) and M2 macrophages ($P = 0.032$) (Fig. 3b). Relations between PUFAs metabolism score and 5 genes coding pivotal immune checkpoints were then analyzed. These 5 genes are PDCD1 (coding PD-1), CD274 (coding PD-L1), LAG3 (coding LAG-3), HAVCR2 (coding TIM-3) and CTLA4 (coding CTLA-4). They were picked for their complementary functions in compromising anti-tumor immunity[35–38]. Results showed that both ω-3 and ω-6 PUFAs metabolism score were positively correlated with expression of PD-L1, LAG-3 and TIM-3 in TIME (all $P < 0.001$). Conversely, ω-6/ω-3 PUFAs metabolism score was negatively correlated with expression of LAG-3 ($P = 0.044$), TIM-3 ($P = 0.020$) and CTLA-4 ($P = 0.042$), although the correlations were relatively weak (Fig. 3c). The subsequently plotted protein–protein interaction (PPI) network contained eighty-seven important proteins from four crucial inflammatory pathways in gastric cancer[39–43], namely the NF-κB pathway, the ras-raf-MAPK pathway, the PI3K-AKT pathway and the JAK-STAT pathway. The PPI network consisted of 117 nodes (representing proteins) and 1206 edges (representing interactions) ($P < 0.05$). Several key proteins were identified through topological analysis of the PPI network. PTGS2 can interact directly with 11 out of 12 key proteins in four inflammation pathways. PTGS1 can interact directly with IL-6 and CXCL8. ALOX5 can interact directly with STAT3, IL-6 and CXCL8. PLA2G4A can interact directly with IL-6, MAPK3 and STAT3. The sub-network of PUFAs related proteins were also connected with several core inflammation proteins such as IL-6 and CXCL8 (Fig. 3d).

Mass spectrometry (MS) determined Serum PUFAs and PD-1/PD-L1 expression

Seventy patients were divided into two groups according to their PD-1/PD-L1 expression levels. Results showed that only two of determined fatty acids had significant differences between high group (n = 11, CPS ≥ 10 or TPS ≥ 10%) and low group (n = 59, CPS < 10 or TPS < 10%), which were AA (5.452 μmol/L vs. 7.315 μmol/L, T test, $P = 0.010$) and C16:1 (9.582 μmol/L vs. 16.854 μmol/L, T test, $P = 0.018$).

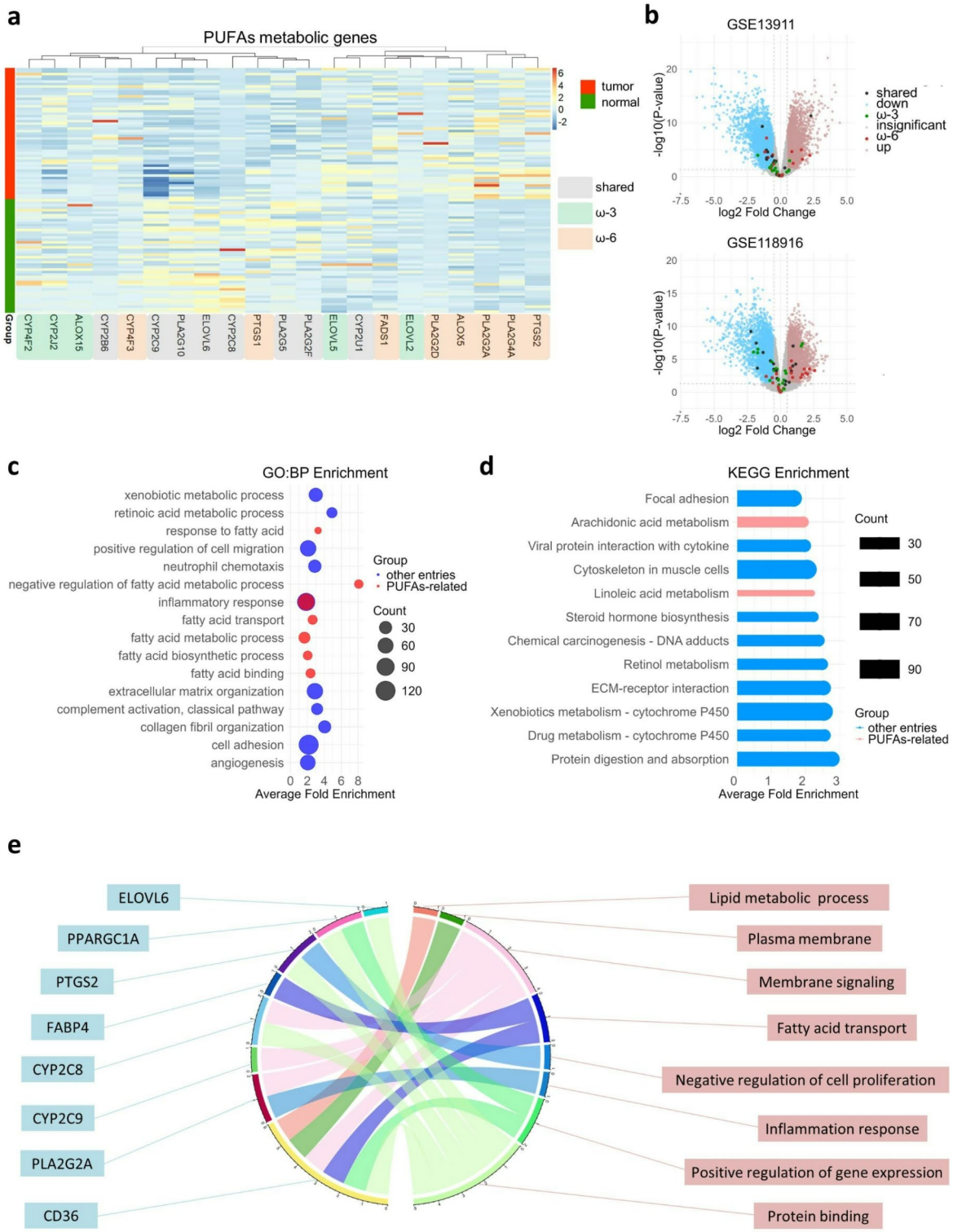


Fig. 2. DEGs and enrichemnt (**a**) Combined PUFAs related genes heatmap of two datasets. (**b**) Expression volcano plot of DEGs of GSE13911 and GSE118916, respectively. (**c**) Enrichment degree of all categories of fatty acids metabolism related genes in GO:BP. Highlighted entries are related to PUFAs metabolism and blue entries are other top 9 significantly enriched biological processes. (**d**) Enrichment degree of polyunsaturated fatty acids related genes in KEGG. Red entries are related to PUFAs metabolism and blue entries are other top 10 enriched pathways ranked by significance. (**e**) Chord plot of crosstalk between PUFAs related genes, inflammation and cellular signaling.

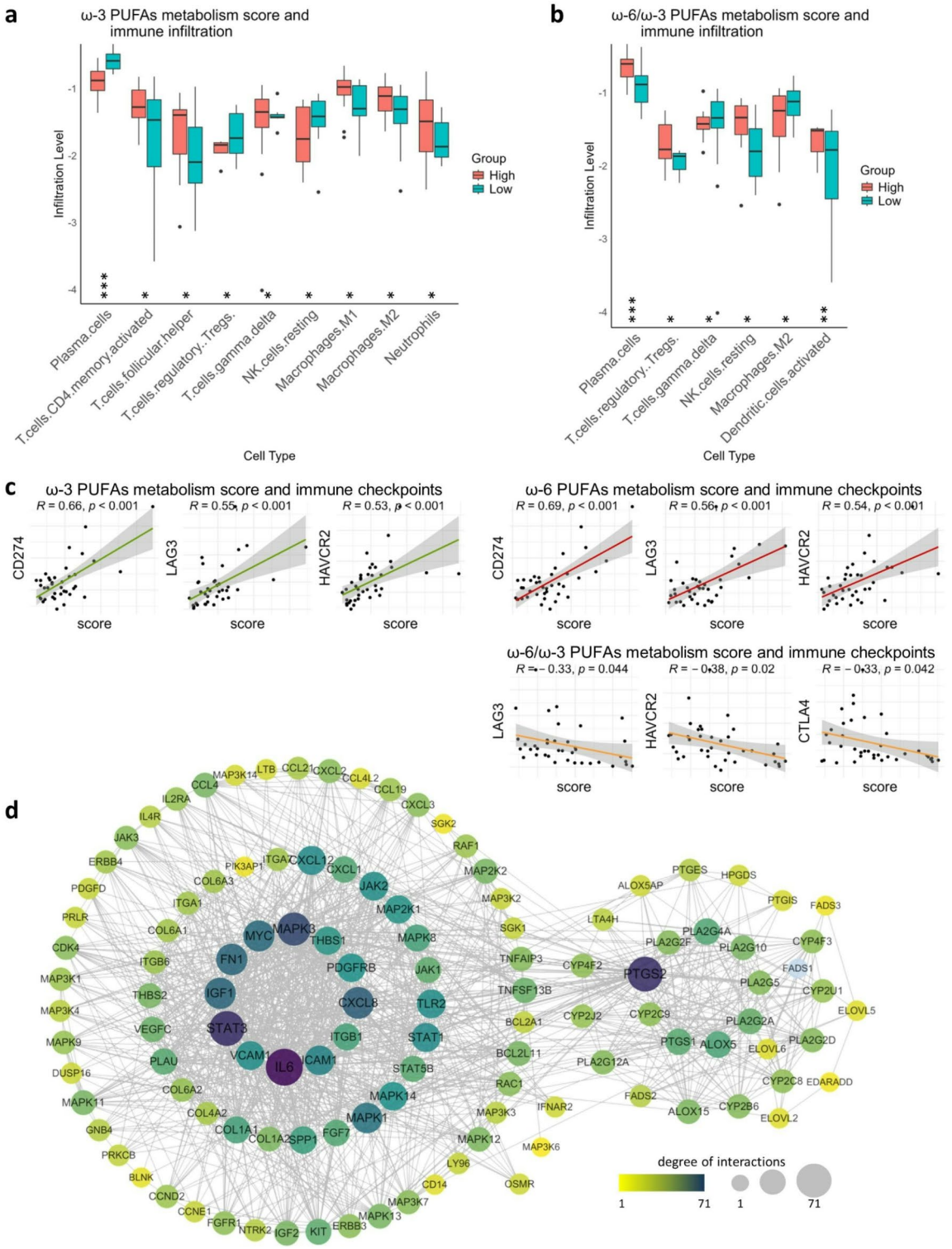


Fig. 3. PUFAs and immune infiltration (**a**, **b**) ω-3 and ω-6/ω-3 PUFAs metabolism score and immune cell infiltration, respectively. Infiltration scales are transformed by log10 calculation. Significance markers: "*": $P < 0.05$; "**": $P < 0.01$; "***": $P < 0.001$. (**c**) Relations between ω-3, ω-6 and ω-6/ω-3 PUFAs metabolism score and immune checkpoints expressions in gastric cancer TME, respectively. (**d**) PPI network of protein Interactions between PUFAs metabolic enzymes and four inflammation pathways (NF-κB, ras-raf-MAPK, PI3K-AKT and JAK-STAT).

Mass spectrometry (MS) determined Serum PUFAs and short-term therapeutic efficacy

Relationships between all determined serum PUFA values, designed ratios, disease control rate (DCR), and objective response rate (ORR) were analyzed (Relation heatmap of determined PUFAs are presented in Fig. 4a; Patients' clinical characteristics are presented in Table 2). Univariate analysis showed that DCR in the high groups of serum oleic acid (OA, 65.7% vs. 88.6%, Chi-Square test, $P=0.046$), AA/EPA ratio (67.1% vs. 87.1%, Chi-Square test, $P=0.045$) and ω-6/ω-3 ratio (65.7% vs. 88.6%, Chi-Square test, $P=0.046$) was lower than that in the low groups. ORR in the high groups of serum AA/EPA ratio (17.1% vs. 45.7%, Chi-Square test, $P=0.020$) and ω-6/ω-3 ratio (17.1% vs. 45.7%, Chi-Square test, $P=0.020$) was lower than that in the low groups. Multivariate analysis indicated that the serum OA (odds ratio [OR] = 0.99, $P=0.005$), the ω-6/ω-3 ratio (odds ratio [OR] = 0.82, $P=0.015$) and the EPA (odds ratio [OR] = 30.46, $P=0.009$) were associated with DCR. The serum AA/EPA ratio (odds ratio [OR] = 0.80, $P=0.018$) and the GLA (odds ratio [OR] = 0.14, $P=0.029$) were associated with ORR. (Fig. 4b).

Mass spectrometry (MS) determined Serum PUFAs and long-term therapeutic efficacy

Relationships between all determined PUFAs' values and designed ratios with disease progression-free survival (PFS) and one year overall survival rate (1-year OS) were analyzed. ECOG score (hazard ratio [HR] 95% CI 1.09 ~ 3.50) and history of radiotherapy (hazard ratio [HR] 95% CI 1.32 ~ 16.00) were independent risk factors for disease progression among all clinical characteristics. Univariate and multivariate Cox regression analysis revealed that, the LA/ALA ratio (hazard ratio [HR] = 1.02, $P=0.023$), the AA/EPA ratio (hazard ratio [HR] = 1.09, $P<0.001$) and the EPA (hazard ratio [HR] = 0.50, $P=0.028$) were correlated with PFS. These results indicated that the serum AA/EPA is an independent risk factor for disease progression, and the serum EPA is an independent protective factor for disease progression. Survival curves based on PFS manifested that only the serum AA/EPA ratio and the EPA had significant correlations with survival outcomes (Log Rank test. $P=0.021$, $P=0.018$, respectively). The serum ω-6/ω-3 ratio, the LA/ALA ratio, the AA/DHA ratio and the GLA only had potential correlations with PFS within the initial approximately 400 days. In terms of one year overall survival, only ECOG score was an independent risk factor (OR 95% CI 0.07 ~ 0.71) among all clinical characteristics. Univariate analysis showed that higher LA/ALA (25.7% vs. 60.0%, Chi-Square test, $P=0.008$) and AA/EPA ratios (20.0% vs. 65.7%, Chi-Square test, $P<0.001$) were associated with lower survival rates while higher EPA levels correlated with improved survival (60.0% vs. 25.7%, $P=0.008$). Multivariate Cox regression indicated that AA/EPA (odds ratio [OR] = 0.51, $P<0.001$) and EPA (odds ratio [OR] = 7.22, $P=0.023$) were independently related to 1-year OS. (Fig. 4b,c).

Predicative values on immunotherapy efficacy

PUFAs values and ratios determined at the beginning of immunotherapy were analyzed for their potential predictive value on immunotherapy efficacy. Results showed that the serum AA/EPA ratio (AUC = 0.69, $P=0.006$) and the EPA (AUC = 0.66, $P=0.024$) had moderate predictive value on ORR. The serum ω-6/ω-3 ratio (AUC = 0.73, $P=0.002$), the AA/EPA ratio (AUC = 0.73, $P<0.001$), the OA (AUC = 0.67, $P=0.030$) and the EPA (AUC = 0.76, $P<0.001$) had moderate predictive value on DCR. The serum ω-6/ω-3 ratio (AUC = 0.68, $P=0.006$) had moderate predictive value on 6-month PFS, while the serum AA/EPA ratio (AUC = 0.84, $P<0.001$) and the EPA (AUC = 0.85, $P<0.001$) had strong predictive value on 6-month PFS. Moreover, the serum AA/EPA ratio (AUC = 0.8, $P<0.001$) had strong predictive value on 1-year OS. The serum LA/ALA ratio (AUC = 0.64, $P=0.048$) and the EPA (AUC = 0.73, $P<0.001$) only had moderate predictive value on 1-year OS. (Fig. 4d and Table 3).

Discussion

Gastric cancer remains a leading cause of cancer-related deaths globally. Despite the significant potential of immunotherapy, its efficacy often fails to align consistently with existing biomarkers. Moreover, current response rates remain suboptimal, underscoring the need for a more profound understanding of the tumor immune microenvironment. PUFAs metabolism plays a pivotal role among diet, inflammation and carcinogenesis. Its importance in shaping the immune landscape of gastric cancer has been underestimated, especially in the era of immunotherapy.

Our study revealed distinct expression patterns of ω-3 and ω-6 PUFAs metabolism-related genes in gastric cancer, which are closely associated with the immune microenvironment. It was found that PLA2 family and CYP family, which are common genes in both ω-3 and ω-6 PUFAs metabolism at the initial stage, are mostly down-regulated in gastric tumor tissue. With the progress of metabolic processes, the direction towards ω-6 PUFAs becomes predominant, which entails the accumulation of downstream metabolites mediating chronic inflammation (e.g., LTs, TXs, and PGs). In the part of immune infiltration analysis, the application of the CIBERSORT method and an experimental tool "PUFAs metabolism score" offered new perspectives: PUFAs metabolism towards ω-3 is linked with higher infiltration of $CD4^+$ memory T cells, follicular Th cells and γδ T cells, and lower infiltration of regulatory T cells. Such immune landscape is overall beneficial in anti-tumor cellular and humoral immunity[44,45]. Conversely, higher ω-6/ω-3 PUFAs metabolism score leads to a harmful immune microenvironment, with higher infiltration of regulatory T cells, resting NK cells[46] and resting dendritic cells[47], and lower infiltration of γδ T cells. Additionally, both ω-3 and ω-6 PUFAs metabolism score are positively correlated with expression of PD-L1, LAG-3 and TIM-3 while ω-6/ω-3 PUFAs metabolism score is negatively correlated with expression of LAG-3, TIM-3 and CTLA-4 in TIME. Therefore, it can be presumed that PUFAs metabolism towards ω-3 may shape an advantageous TIME. In contrast, PUFAs metabolism towards ω-6, especially with a higher ω-6/ω-3 ratio of metabolic direction, is largely detrimental. According to our further analysis of protein–protein interactions, these gastric TIME features likely arise from a sophisticated crosstalk between PUFAs metabolism and inflammatory pathways, orchestrated by three interdependent mechanisms:

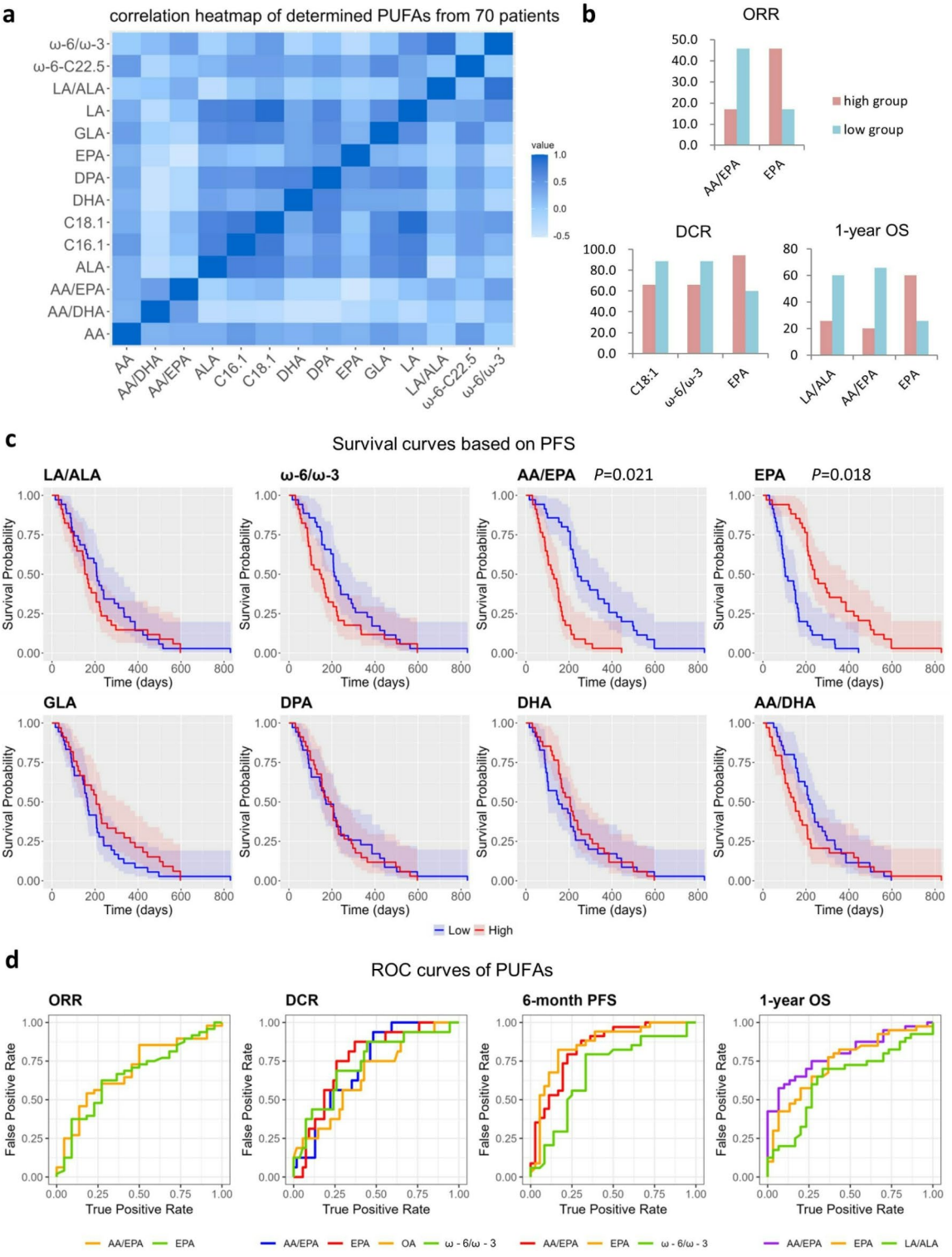


Fig. 4. PUFAs and immunotherapy (**a**) correlation heatmap of determined unsaturated poly fatty acids from 70 patients. Considering multicollinearity (multicollinearity test, VIF > 10, $P < 0.05$), the source substrates of the two metabolic directions, LA and ALA, were separately analyzed. (**b**) High and low serum PUFAs level groups and therapeutic efficacy. Only those whose significance less than 0.05 are presented. (**c**) Survival curves based on PFS of high groups and low groups. (**d**) ROC curves of PUFAs' predictive values on immunotherapy efficacy and survival. Only those whose significance less than 0.05 are presented.

Clinical characteristics	Number of patients N = 70	P (ORR)	P (DCR)	P (PFS)	P (1-year OS)
Age, years		0.382	0.508	0.809	0.502
≥ 65	41 (58.57%)				
< 65	29 (41.43%)				
Sex		0.552	0.657	0.316	0.795
Male	54 (77.14%)				
Female	16 (22.86%)				
Stage		0.999	0.999	0.514	0.999
III	2 (2.94%)				
IV	68 (97.06%)				
ECOG[1]		0.533	0.258	**0.025**	**0.013**
0	26 (37.14%)				
1	44 (62.86%)				
History of radiotherapy	0.999	0.999	0.662	**0.017**	0.663
Yes	3 (4.29%)				
No	67 (95.71%)				
Treatment line		0.321	0.144	0.792	0.723
1	38 (54.29%)				
2	29 (41.43%)				
≥ 3	3 (4.29%)				
History of smoking		0.699	0.183	0.950	0.147
Yes	18 (25.71%)				
No	52 (74.29%)				
Immunotherapy indications[2]	0.987	0.987	0.060	0.187	0.983
Yes	19 (27.14%)				
No	51 (72.86%)				
ICBs[3]		0.370	0.681	0.756	0.965
Sintilimab	37 (52.86%)				
Nivolumab	9 (12.86%)				
Camrelizumab	5 (7.14%)				
Toripalimab	3 (4.29%)				
Pembrolizumab	2 (2.86%)				
Tislelizumab	10 (14.29%)				
Penpulimab	4 (5.71%)				

Table 2. Clinical baseline characteristics of 70 patients. [a]ECOG refers to physical condition score designed by Eastern Cooperative Oncology Group. [b]immunotherapy indications refer to dMMR/MSI-H/PD-L1 CPS > 1/ PD-L1 TPS > 1%/TMB > 10. [c]ICBs stands for immune checkpoints blockers. Significant values are in bold.

(1) PTGS2 (COX-2) serves as the central hub, interacting with 11 out of 12 inflammatory proteins to bridge ω-6 PUFAs conversion to multifarious inflammatory mediators; (2) PTGS1 (COX-1) and ALOX5 (5-LOX) exhibit pathway-specific modulation. PGE2 derived from COX-1 amplifies IL-6 via EP2/EP4-STAT3, while 5-LOX-generated LTB4 enhances CXCL8 through BLT1-Rac1; (3) PLA2 family fuels this network by supplying AA substrate, creating a feed-forward loop that converges on MAPK3/STAT3 activation. Moreover, the co-presence of other PUFAs metabolic enzymes (e.g., ALOX15 for resolvins, PTGIS for prostacyclin) suggests a self-regulating system, where pro-inflammatory drivers are counterbalanced by specialized pro-resolving mediators. During the design of the metabolic score, although we rigorously referred to a large number of literatures in an attempt to be as scientific and reasonable as possible, it was still difficult to determine the actual weights of each gene due to the complexity of biological systems. However, what is gratifying is that the designed score is closely related to the tumor immune microenvironment. This association strongly attests to the rationality of the score design. At the same time, it also paves the way for more in-depth and extensive related research in the future.

Our findings also highlight the potential of serum PUFAs levels as an economical and effective biomarker for predicting immunotherapy outcomes. Based on findings by bioinformatics and existing literature reviews, we speculate that PUFAs landscape in gastric cancer depends on two aspects, which are enzyme aberrations and proportions of substrates. The latter can be influenced by different dietary habits and non-tumor mediated inflammation. Based on such consideration, this study established rigorous restrictions on patients' physical performance scores, enteral and parenteral nutrition usage and various factors that may contribute to inflammation. Serum PUFAs should remain stable with these restrictions according to previous studies[48,49]. By sorting out the metabolic process of PUFAs, we designed some ratios (e.g., LA/ALA, AA/EPA and AA/

	PUFAs (all $P<0.05$)	Cutoff values[a]	AUC	Sensitivity (%)	Specificity (%)
ORR[b]	AA/EPA	12.00	0.69	81.8	54.2
	EPA	0.704	0.66	72.7	62.5
DCR[c]	ω-6/ω-3	12.00	0.73	55.6	87.5
	EPA	0.638	0.76	63.0	87.5
	OA	186.0	0.67	57.4	75.0
	AA/EPA	9.11	0.73	51.9	93.8
6-month PFS[d]	ω-6/ω-3	11.80	0.68	69.7	79.4
	AA/EPA	9.11	0.84	69.4	88.2
	EPA	0.638	0.85	83.3	82.4
1-year OS[e]	AA/EPA	13.70	0.80	93.3	57.5
	LA/ALA	20.90	0.64	66.7	70.0
	EPA	0.704	0.74	63.3	77.5

Table 3. PUFAs' predictive value on immunotherapy efficacy and survival. [a]The unit of serum polyunsaturated fatty acids is μmol/L. Ratios have no units. [b]*ORR* objective response rate. [c]*DCR* disease control rate. [d]*6-month PFS* six-month progression-free survival rate. [e]*1-year OS* one-year overall survival rate.

DHA) for further analysis. The source substrates of the two metabolic directions, LA and ALA, were separately analyzed considering their multicollinearity with other PUFAs. With mass spectrometry as an accurate and highly sensitive quantitative analysis tool[50–52], our study innovatively applied the PUFAs network to predict the efficacies of clinical gastric cancer immunotherapy. It was found that high serum ω-6 PUFAs (e.g., LA,GLA, ω-6-DPA and AA) are unlikely to lower ORR, DCR, PFS, or 1-year OS, but higher serum LA/ALA ratio (initial substrate of ω-6 and ω-3 PUFAs), total ω-6/ω-3 PUFAs ratio, and AA/EPA ratio (active metabolites of ω-6 and ω-3 PUFAs) are likely to compromise both short-term and long-term survival. Higher serum EPA level is found to be significantly beneficial for both short-term and long-term efficacy, indicating EPA's higher activity in TME inflammation regulation. Currently, most popular biomarkers for predicting immunotherapy efficacy in gastric cancer include PD-1/PD-L1, MMR status, MSI status and TMB. Accurate detections of such genetic aberrations, however, rely on adequate tumor sampling and expensive genetic testing. Hence, our empirically built predictive framework based on serum PUFAs would be economical and promising in guiding individualized therapeutic strategies. It is therefore recommended that clinical strategies consider modulating PUFAs metabolism to enhance anti-tumor immune responses, which can be potentially achieved through specific dietary adjustments or pharmacological interventions.

Our research contributes to the existing body of knowledge in several ways. First, rather than focusing solely on single genes, we have conducted a comprehensive and in-depth analysis of the PUFAs metabolic network in gastric cancer, reducing the potential for bias that may arise from overemphasis on individual genetic elements. Second, we developed a tool named "PUFAs metabolic score" and integrated it with the CIBERSORT method. Through this combination, we were able to uncover previously unreported relationships between PUFAs and the immune landscape in gastric cancer. In addition, by employing mass spectrometry, we were able to accurately measure different serum PUFAs.

The study's limitations due to funding and technological constraints highlight the need for further research on a larger scale to validate the clinical applicability of the PUFAs metabolism score. Future studies combining bioinformatics, pharmacology, and immunology should expand sample sizes and delve deeper into the interactions between PUFAs metabolism and TIME, as well as how these interactions affect treatment outcomes in vitro and in vivo to expect more comprehensive and innovative solutions for gastric cancer.

Data availability

The bioinformatics data that support the findings of this study are available from Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The clinical data that support the findings of this study are collected from Zhongda Hospital, Southeast University, but restrictions apply to the availability of these data, which are not publicly available. The data are however available from the corresponding author on reasonable request.

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References

1. Thrift, A. P., Wenker, T. N. & El-Serag, H. B. Global burden of gastric cancer: Epidemiological trends, risk factors, screening and prevention. *Nat. Rev. Clin. Oncol.* **20**(5), 338–349. https://doi.org/10.1038/s41571-023-00747-0 (2023).
2. Guan, W. L., He, Y. & Xu, R. H. Gastric cancer treatment: Recent progress and future perspectives. *J. Hematol. Oncol.* **16**(1), 57. https://doi.org/10.1186/s13045-023-01451-3 (2023).
3. Zhang, Y. & Zhang, Z. The history and advances in cancer immunotherapy: Understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol. Immunol.* **17**(8), 807–821. https://doi.org/10.1038/s41423-020-0488-6 (2020).
4. Kang, B. W. & Chau, I. Current status and future potential of predictive biomarkers for immune checkpoint inhibitors in gastric cancer. *ESMO Open* **5**(4), e000791. https://doi.org/10.1136/esmoopen-2020-000791 (2020).

5. Catalano, M. et al. Immunotherapy-related biomarkers: Confirmations and uncertainties. *Crit. Rev. Oncol. Hematol.* **192**, 104135. https://doi.org/10.1016/j.critrevonc.2023.104135 (2023).
6. Li, K. et al. Advances in clinical immunotherapy for gastric cancer. *Biochim. Biophys. Acta Rev. Cancer* **2**, 188615. https://doi.org/10.1016/j.bbcan.2021.188615(2021) (1876).
7. Kapoor, B. et al. Dietary polyunsaturated fatty acids (PUFAs): Uses and potential health benefits. *Curr. Nutr. Rep.* **10**(3), 232–242. https://doi.org/10.1007/s13668-021-00363-3 (2021).
8. Harwood, J. L. Polyunsaturated fatty acids: Conversion to lipid mediators, roles in inflammatory diseases and dietary sources. *Int. J. Mol. Sci.* **24**(10), 8838. https://doi.org/10.3390/ijms24108838 (2023).
9. Huang, F., Wang, K. & Shen, J. Lipoprotein-associated phospholipase A2: The story continues. *Med. Res. Rev.* **40**(1), 79–134. https://doi.org/10.1002/med.21597 (2020).
10. Wang, B. et al. Metabolism pathways of arachidonic acids: Mechanisms and potential therapeutic targets. *Signal Transduct. Target Ther.* **6**(1), 94. https://doi.org/10.1038/s41392-020-00443-w (2021).
11. Brayner, B., Kaur, G., Keske, M. A. & Livingstone, K. M. FADS polymorphism, Omega-3 fatty acids and diabetes risk: A systematic review. *Nutrients* **10**(6), 758. https://doi.org/10.3390/nu10060758 (2018).
12. Loukil, I., Mutch, D. M. & Plourde, M. Genetic association between FADS and ELOVL polymorphisms and the circulating levels of EPA/DHA in humans: A scoping review. *Genes Nutr.* **19**(1), 11. https://doi.org/10.1186/s12263-024-00747-4 (2024).
13. Barker, H. E., Cox, T. R. & Erler, J. T. The rationale for targeting the LOX family in cancer. *Nat. Rev. Cancer* **12**(8), 540–552. https://doi.org/10.1038/nrc3319 (2012).
14. Gao, Z. et al. Effects of omega-3 polyunsaturated fatty acids on coronary atherosclerosis and inflammation: A systematic review and meta-analysis. *Front. Cardiovasc. Med.* **9**, 904250. https://doi.org/10.3389/fcvm.2022.904250 (2022).
15. Mizuno, R., Kawada, K. & Sakai, Y. Prostaglandin E2/EP signaling in the tumor microenvironment of colorectal cancer. *Int. J. Mol. Sci.* **20**(24), 6254. https://doi.org/10.3390/ijms20246254 (2019).
16. Ulven, T. & Christiansen, E. Dietary fatty acids and their potential for controlling metabolic diseases through activation of FFA4/GPR120. *Ann. Rev. Nutr.* **35**(35), 239–263. https://doi.org/10.1146/annurev-nutr-071714-034410 (2015).
17. Korbecki, J., Bobinski, R. & Dutka, M. Self-regulation of the inflammatory response by peroxisome proliferator-activated receptors. *Inflamm. Res.* **68**(6), 443–458. https://doi.org/10.1007/s00011-019-01231-1 (2019).
18. Liput, K. P. et al. Effects of dietary n-3 and n-6 polyunsaturated fatty acids in inflammation and cancerogenesis. *Int. J. Mol. Sci.* **22**(13), 6965. https://doi.org/10.3390/ijms22136965 (2021).
19. Viswanathan, V. S. et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. *Nature* **547**(7664), 453–457. https://doi.org/10.1038/nature23007 (2017).
20. de van der Schueren, M. A. E. et al. Systematic review and meta-analysis of the evidence for oral nutritional intervention on nutritional and clinical outcomes during chemo(radio)therapy: Current evidence and guidance for design of future trials. *Ann. Oncol.* **29**(5), 1141–1153. https://doi.org/10.1093/annonc/mdy114 (2018).
21. Wang, D., Cabalag, C. S., Clemons, N. J. & DuBois, R. N. Cyclooxygenases and prostaglandins in tumor immunology and microenvironment of gastrointestinal cancer. *Gastroenterology* **161**(6), 1813–1829. https://doi.org/10.1053/j.gastro.2021.09.059 (2021).
22. Wei, J. et al. The COX-2-PGE2 pathway promotes tumor evasion in colorectal adenomas. *Cancer Prev. Res. (Phila).* **15**(5), 285–296. https://doi.org/10.1158/1940-6207.CAPR-21-0572 (2022).
23. Xiang, W. et al. Inhibition of ACLY overcomes cancer immunotherapy resistance via polyunsaturated fatty acids peroxidation and cGAS-STING activation. *Sci. Adv.* **9**(49), eadi2465. https://doi.org/10.1126/sciadv.adi2465 (2023).
24. Pope, L. E. & Dixon, S. J. Regulation of ferroptosis by lipid metabolism. *Trends Cell Biol.* **33**(12), 1077–1087. https://doi.org/10.1016/j.tcb.2023.05.003 (2023).
25. Oya, Y., Hayakawa, Y. & Koike, K. Tumor microenvironment in gastric cancers. *Cancer Sci.* **111**(8), 2696–2707. https://doi.org/10.1111/cas.14521 (2020).
26. Wang, B., Zhang, Z., Liu, W. & Tan, B. Targeting regulatory T cells in gastric cancer: Pathogenesis, immunotherapy, and prognosis. *Biomed. Pharmacother.* **158**, 114180. https://doi.org/10.1016/j.biopha.2022.114180 (2023).
27. Yin, S. et al. The prognostic and clinicopathological significance of tumor-associated macrophages in patients with gastric cancer: A meta-analysis. *PLoS ONE* **12**(1), e0170042. https://doi.org/10.1371/journal.pone.0170042 (2017).
28. Sautes-Fridman, C., Petitprez, F., Calderaro, J. & Fridman, W. H. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat. Rev. Cancer* **19**(6), 307–325. https://doi.org/10.1038/s41568-019-0144-6 (2019).
29. Fu, Z. et al. Construction of miRNA-mRNA-TF regulatory network for diagnosis of gastric cancer. *Biomed. Res. Int.* **2021**, 9121478. https://doi.org/10.1155/2021/9121478 (2021).
30. Han, S. et al. miR-29a-3p-dependent COL3A1 and COL5A1 expression reduction assists sulforaphane to inhibit gastric cancer progression. *Biochem. Pharmacol.* **188**, 114539. https://doi.org/10.1016/j.bcp.2021.114539 (2021).
31. Wang, Y. et al. Bioinformatics analysis identifies COL1A1, THBS2 and SPP1 as potential predictors of patient prognosis and immunotherapy response in gastric cancer. *Biosci. Rep.* **41**(1), BSR20202564. https://doi.org/10.1042/BSR20202564 (2021).
32. Wang, R. et al. Identification of key genes with prognostic value in gastric cancer by bioinformatics analysis. *Front. Genet.* **13**, 958213. https://doi.org/10.3389/fgene.2022.958213 (2022).
33. Pei, X. et al. Identification and validation of key genes in gastric cancer: Insights from in silico analysis, clinical samples, and functional assays. *Aging (Albany NY)* **16**(12), 10615–10635. https://doi.org/10.18632/aging.205965 (2024).
34. Kanehisa, M. et al. Biological systems database as a model of the real world. *Nucleic Acids Res.* **53**(D1), D672–D677. https://doi.org/10.1093/nar/gkae909 (2025).
35. Hosseini, A. et al. CTLA-4: From mechanism to autoimmune therapy. *Int. Immunopharmacol.* **80**, 106221. https://doi.org/10.1016/j.intimp.2020.106221 (2020).
36. Tang, Q. et al. The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers. *Front. Immunol.* **13**, 964442. https://doi.org/10.3389/fimmu.2022.964442 (2022).
37. Das, M., Zhu, C. & Kuchroo, V. K. Tim-3 and its role in regulating anti-tumor immunity. *Immunol. Rev.* **276**(1), 97–111. https://doi.org/10.1111/imr.12520 (2017).
38. Chocarro, L. et al. Understanding LAG-3 Signaling. *Int. J. Mol. Sci.* **22**(10), 5282. https://doi.org/10.3390/ijms22105282 (2021).
39. Yu, H. et al. Targeting NF-kappaB pathway for the therapy of diseases: Mechanism and clinical study. *Signal Transduct. Target Ther.* **5**(1), 209. https://doi.org/10.1038/s41392-020-00312-6 (2020).
40. Wang, C. et al. Macrophage polarization and its role in liver disease. *Front. Immunol.* **12**, 803037. https://doi.org/10.3389/fimmu.2021.803037 (2021).
41. Ullah, R., Yin, Q., Snell, A. H. & Wan, L. RAF-MEK-ERK pathway in cancer evolution and treatment. *Semin. Cancer Biol.* **85**, 123–154. https://doi.org/10.1016/j.semcancer.2021.05.010 (2022).
42. Fan, Y., Mao, R. & Yang, J. NF-kappaB and STAT3 signaling pathways collaboratively link inflammation to cancer. *Protein Cell.* **4**(3), 176–185. https://doi.org/10.1007/s13238-013-2084-3 (2013).
43. Acosta-Martinez, M. & Cabail, M. Z. The PI3K/Akt pathway in meta-inflammation. *Int. J. Mol. Sci.* **23**(23), 15330. https://doi.org/10.3390/ijms232315330 (2022).
44. Cui, C., Craft, J. & Joshi, N. S. T follicular helper cells in cancer, tertiary lymphoid structures, and beyond. *Semin. Immunol.* **69**, 101797. https://doi.org/10.1016/j.smim.2023.101797 (2023).

45. Mensurado, S., Blanco-Dominguez, R. & Silva-Santos, B. The emerging roles of gammadelta T cells in cancer immunotherapy. *Nat. Rev. Clin. Oncol.* **20**(3), 178–191. https://doi.org/10.1038/s41571-022-00722-1 (2023).
46. Melaiu, O., Lucarini, V., Cifaldi, L. & Fruci, D. Influence of the tumor microenvironment on NK cell function in solid tumors. *Front. Immunol.* **10**, 3038. https://doi.org/10.3389/fimmu.2019.03038 (2019).
47. Del Prete, A. et al. Dendritic cell subsets in cancer immunity and tumor antigen sensing. *Cell Mol. Immunol.* **20**(5), 432–447. https://doi.org/10.1038/s41423-023-00990-6 (2023).
48. Lelli, D. et al. Association between PUFA intake and serum concentration and mortality in older adults: A cohort study. *Clin. Nutr.* **39**(2), 510–515. https://doi.org/10.1016/j.clnu.2019.02.030 (2020).
49. Bartha, V. et al. Changes in serum omega fatty acids on a Mediterranean diet intervention in patients with gingivitis: An exploratory study. *J. Periodontal Res.* **57**(6), 1198–1209. https://doi.org/10.1111/jre.13056 (2022).
50. Buchberger, A. R., DeLaney, K., Johnson, J. & Li, L. Mass spectrometry imaging: A review of emerging advancements and future insights. *Anal. Chem.* **90**(1), 240–265. https://doi.org/10.1021/acs.analchem.7b04733 (2018).
51. Holzlechner, M., Eugenin, E. & Prideaux, B. Mass spectrometry imaging to detect lipid biomarkers and disease signatures in cancer. *Cancer Rep. (Hoboken)* **2**(6), e1229. https://doi.org/10.1002/cnr2.1229 (2019).
52. Hu, T. & Zhang, J. L. Mass-spectrometry-based lipidomics. *J. Sep. Sci.* **41**(1), 351–372. https://doi.org/10.1002/jssc.201700709 (2018).

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Author contributions
Haijun Zhang provided overall direction for the project. Dikan Wu was responsible for bioinformatics analysis, plotting, clinical data collection, data analysis and preparation of the manuscript. JunXian He provided technical support on bioinformatics. Fan Fan, Lian Wang, Caiyun Zhu, Yalin Ji participated in interpreting the information, editing, and critically revising the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethical statement
In the retrospective clinical study, all methods were carried out in accordance with relevant medical research guidelines and regulations. Patients' blood samples were collected for multiple clinical diagnosis and treatment purposes with the consent of Zhongda Hospital, Southeast University. We confirm that informed consents were obtained from all patients and/or their legal guardians. The study was approved by the Ethics Committee of Zhongda Hospital, Southeast University.

Consent to publish
All authors of this study agreed with the publication.

Additional information
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