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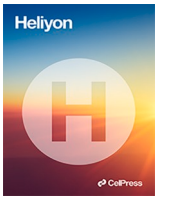

Lipid metabolism-derived FAAH is a sensitive marker for the prognosis and immunotherapy of osteosarcoma patients

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A B S T R A C T

Lipid metabolism in cancer refers to the alterations in how cancer cells process and utilize lipids, a type of fat molecule. It was investigated how lipid metabolism relates to osteosarcoma. Genes relevant to lipid metabolism were gathered to create lipid metabolism-associated clusters and locate the dangerous marker. We investigated FAAH's prognostic significance, route annotation, immunotherapy response, and medication prediction. Besides, FAAH is proven to be a potent, dangerous marker that may promote growth and migration and inhibit the apoptosis of osteosarcoma. FAAH exhibits higher expression levels in tumor tissues as compared to normal tissues. In conclusion, FAAH is identified in this work as a potentially dangerous gene and immunotherapy determinant. This study requires more investigation to determine how FAAH influences the immune response in osteosarcoma.

1. Introduction

An uncommon form of cancer called osteosarcoma (OS) develops from the mesenchymal cells that make up bones. In children and adolescents, it is the most common and widely spreading bone tumor [1]. With a second peak occurring in older individuals, the prevalence of this condition is higher among adolescents, with a rate of 0.8–1.1 cases per 100,000 individuals per year in the age group of 15–19 years [2,3]. The bones near the knee joint that are most commonly affected are the distal femur, proximal humerus, and proximal tibia. These three bones account for over two-thirds of primary tumors in that area [4]. Despite the vast genomic abnormalities, OS lacks targetable mutations or pathognomonic DNA translocations [5]. As a result, there are currently no efficient molecularly targeted therapeutics for OS. The diagnosis of OS is determined solely by morphological criteria since no specific molecular markers or tests are available in clinical practice. OS administration is difficult and necessitates a diverse approach. Standard clinical procedures for OS treatment include surgical excision and methodical multiagent therapy. Due to the high rate of relapse and poor prognosis of metastatic cancer, there is an urgent need to discover new therapeutic approaches and biomarkers to improve the management of the disease.

Lipid metabolism in cancer refers to the alterations in how cancer cells process and utilize lipids, a type of fat molecule. In normal cells, lipid metabolism is tightly regulated to maintain cellular homeostasis. However, in cancer cells, this regulation is often disrupted, leading to changes in lipid synthesis, uptake, storage, and breakdown [6]. Osteosarcoma cells often exhibit increased lipid synthesis, leading to higher levels of fatty acids and cholesterol. Cancer cells then utilize these lipids to support their rapid growth and proliferation. Through the hsa-miR-1184/FADS2 pathway, Hsa_circ_0000073 encourages lipid production in osteosarcoma [7]. HuR-mediated MYC activation is prevented by CircREOS, leading to suppressed lipid production and osteosarcoma development [8].

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Dihydroartemisinin Regulates Loxl2/VEGFA Expression and Lipid Metabolism Pathway to Potentiate the Antitumorigenic Effect of VEGFR-TKI in Osteosarcoma [9].

The tumor microenvironment in osteosarcoma refers to the surrounding cells, blood vessels, and extracellular matrix that interact with the tumor cells [10]. In osteosarcoma, tumor growth, invasion, and response to treatment are influenced by various factors, including the immune system. Immunotherapy, which utilizes the immune system to target and eliminate cancer cells, is being investigated as a potential treatment option for osteosarcoma. Specifically, immune checkpoint inhibitors and adoptive cell therapy are being explored in the context of immunotherapy for this type of cancer [11]. These therapies aim to enhance the immune response against the tumor cells and improve patient outcomes. However, studies exploring the interconnection between lipid metabolism and tumor microenvironment and immunotherapy in osteosarcoma are scarce.

FAAH, also known as fatty acid amide hydrolase, is an enzyme that plays a crucial role in the endocannabinoid system [12]. The system is the endocannabinoid system, which regulates various physiological processes, including pain sensation, mood, appetite, and inflammation. FAAH specifically breaks down anandamide, which is an endocannabinoid that binds to cannabinoid receptors in the body. By breaking down anandamide, FAAH helps to regulate its levels and maintain balance in the endocannabinoid system. However, dysregulation of FAAH activity has been implicated in various pathogenic roles. For example, increased FAAH activity has been associated with reduced levels of anandamide, leading to increased pain sensitivity and inflammation [13]. On the other hand, decreased FAAH activity can result in elevated anandamide levels, which may contribute to mood disorders such as anxiety and depression [14]. Understanding the pathogenic roles of FAAH is important for developing targeted therapies that can modulate its activity and restore balance in the endocannabinoid system. In cancer, FAAH has been shown to regulate the levels of endocannabinoids, which are lipid-signaling molecules [15] that can affect tumor growth and metastasis. Studies have suggested that FAAH may be involved in regulating tumor cell survival, angiogenesis, and immune response in different types of cancer [16]. The specific roles of FAAH in osteosarcoma have not been studied or investigated yet.

In this study, lipid metabolism-related genes were collected. By machine learning algorithm, FAAH was identified as a potent hazardous marker in osteosarcoma. FAAH was further validated to be an immunotherapy determinant. Our study aimed to establish the connection between lipid metabolism and immune activity in osteosarcoma.

1.1. Methods and materials

1.1.1. Data collection

The GSE21257 cohort from the Gene Expression Omnibus (GEO) database and the TARGET cohort from the TARGET database were used in the ensuing research. TARGET database mainly uses molecular characteristics to describe genetic changes in the occurrence and progression of refractory childhood tumors. To date, five diseases, including osteosarcoma, are included in the TARGET tumor database. The TARGET tumor database mainly contains patient clinical data, whole genome sequencing data, methylation data, miRNA data, etc. There were 85 osteosarcoma patients with available clinical characteristics in the TARGET cohort. In agreement with the earlier report, the lipid metabolism-related genes were collected from the Reactome database [17].

1.2. Development of the lipid metabolism-related clusters

We used the TARGET cohort to identify prognostic lipid metabolism-related genes and performed a univariate Cox regression analysis with a significance threshold of $P < 0.001$. We then used the PAM approach from the R package clusterprofiler to identify clusters associated with lipid metabolism. The survival curve between the two lipid metabolism-related clusters was generated using the R package survival. Finally, the prognostic lipid metabolism-related genes between the two clusters were displayed using the R package ComplexHeatmap as a heatmap.

1.3. Functions of the lipid metabolism-related clusters

We used the volcano plot to visualize the differentially expressed genes (DEGs) to annotate the pathways of the two lipid metabolism-related clusters. They then performed GSVA using the KEGG pathways to analyze the DEGs between the two clusters associated with lipid metabolism. The DEGs between two clusters linked to lipid metabolism were examined for enrichment using the Biological Process (BP) pathways.

1.4. Identification of FAAH as a hazardous marker

We performed a multivariate Cox regression analysis to identify other prognostic clinical factors besides FAAH. The Random Survival Forest technique was used for dimension reduction of the data, and the R package survival was used for survival curves comparing the two FAAH-stratified groups.

1.5. Oncogenic and immune analysis of FAAH

Nine common oncogenes were collected from the previous publication [18]. The correlation between FAAH and immune infiltrating cells was based on the ssGSEA algorithm [19]. We also examined the correlation between FAAH and immune modulators. Additionally, they used the ESTIMATE algorithm to calculate the microenvironment scores [20].

1.6. Drug prediction and molecular docking of FAAH

The R package 'oncoPredict' was used to predict the drug sensitivity, similar to the half maximal inhibitory concentration (IC50) [21]. To perform molecular docking between the drug candidate and FAAH, we used AutodockVina 1.2.2 [22]. We retrieved the molecular structures of AZD5991, Dihydrorotenone, PD173074, Sorafenib, and Wnt-C59 from PubChem Compound [23]. The detailed methods for molecular docking are provided in the supplementary file.

1.7. Immunotherapy prediction of FAAH

The GSVA of KEGG pathways was applied to the DEGs between two FAAH-related groups. The software known as Subnetwork Mappings in Alignment of Pathways (Submap) is utilized to predict the results and effectiveness of immunotherapy targeting PD-1 and CTLA-4 in relation to FAAH [24]. Biomarker Exploration of Solid Tumors (BEST) was further used to evaluate the immunotherapy prediction of FAAH.

1.7.1. Cell culture

The cell cultures of U2OS and MNNG/HOS cells were carefully maintained at a constant temperature of 37 °C in a controlled environment with saturated humidity and a 5 % CO2 atmosphere. The culture medium was regularly replaced every 24–48 h to ensure optimal conditions. For cell detachment, Trypsin-EDTA from Gibco in the USA was used, and subculturing was initiated when cell adhesion reached an 80 % confluency threshold. The detailed methods for cell culture are provided in the supplementary file.

1.7.2. siRNA transfection

FAAH siRNA (si-FAAH) and a non-specific control siRNA (si-NC) were synthesized by GenePharma (China). The specific siRNA sequences were designed as follows: FAAH-1 (Forward: GGGACAAGAUGCUGCAGAATT, Reverse: UUCUGCAGCAUCUUGUCCCTT), FAAH-2 (Forward: GGGCUUGAGCCUGAAUGAATT, Reverse: UUCAUUCAGGCUCAAGCCCTT), FAAH-3 (Forward: CCAACUGUGUGACCUCCUATT, Reverse: UAGGAGGUCACACAGUUGGTT), and FAAH-4 (Forward: CCUGAAGGGCUGUGUCUAUTT, Reverse: AUAGACACAGCCCUUCAGGTT). The jetPRIME transfection reagent from Polyplus in France transfected siRNA into U2OS and MNNG/HOS cells. The growth medium was renewed 24 h after transfection, and RNA extraction was conducted 48 h post-transfection.

1.7.3. RT-qPCR

The primer sequences were as follows: FAAH (Forward: GAGACTCAGCTGTCTCAGGC, Reverse: TGGAGTCCTGGCCCTTGTAG), and GAPDH (Forward: ACAGCCTCAAGATCATCAGC, Reverse: GGTCATGAGTCCTTCCACGAT). The detailed methods for RT-qPCR are provided in the supplementary file.

1.7.4. Cell migration assay

To evaluate cell migration, we employed six-well Transwell plates equipped with polycarbonate membranes having an 8 μm pore size (Corning). In the upper chamber, cells were seeded at a density of 1.0×10^6 (500μL/well) using either McCoy's 5A or MEM. The culture medium in the lower chamber was supplemented with 10 % FBS. Cells on the membrane's upper surface were delicately removed by swabbing. Subsequently, a 0.1 % crystal violet stain was administered to the cells, and cell quantification was conducted across six randomly selected fields per well.

1.7.5. Cell proliferation assay

The detailed methods for Cell Counting Kit-8 (CCK-8) are provided in the supplementary file.

1.7.6. EdU assay

The detailed methods for EdU are provided in the supplementary file.

1.7.7. TUNEL staining of the cell

Apoptosis-related staining of U2OS and MNNG cells was carried out using the TUNEL staining kit from Meilunbio in China. The nuclear nuclei were stained with DAPI from Invitrogen in the USA. The quantification of EdU-positive cells was based on Image Pro-Plus version 6.0 from Media Cybernetics in the USA.

1.7.8. Immunohistochemistry

The detailed methods for immunohistochemistry are provided in the supplementary file.

1.7.9. Statistical analysis

R version 3.6.3 (https://www.r-project.org/) was used for all bioinformatics statistical studies and visualization. To assess correlations between continuous variables, Spearman correlation analysis was used. Statistical analyses, the Wilcoxon test and One-way ANOVA, were used to compare group differences based on GraphPad Prism version 8.0.2.263. Statistical significance was determined as a P value less than 0.05, unless otherwise specified.

2. Results

2.1. Development of the lipid metabolism-related clusters

A univariate Cox regression analysis was conducted in the TARGET cohort to identify prognostic lipid metabolism-related genes.

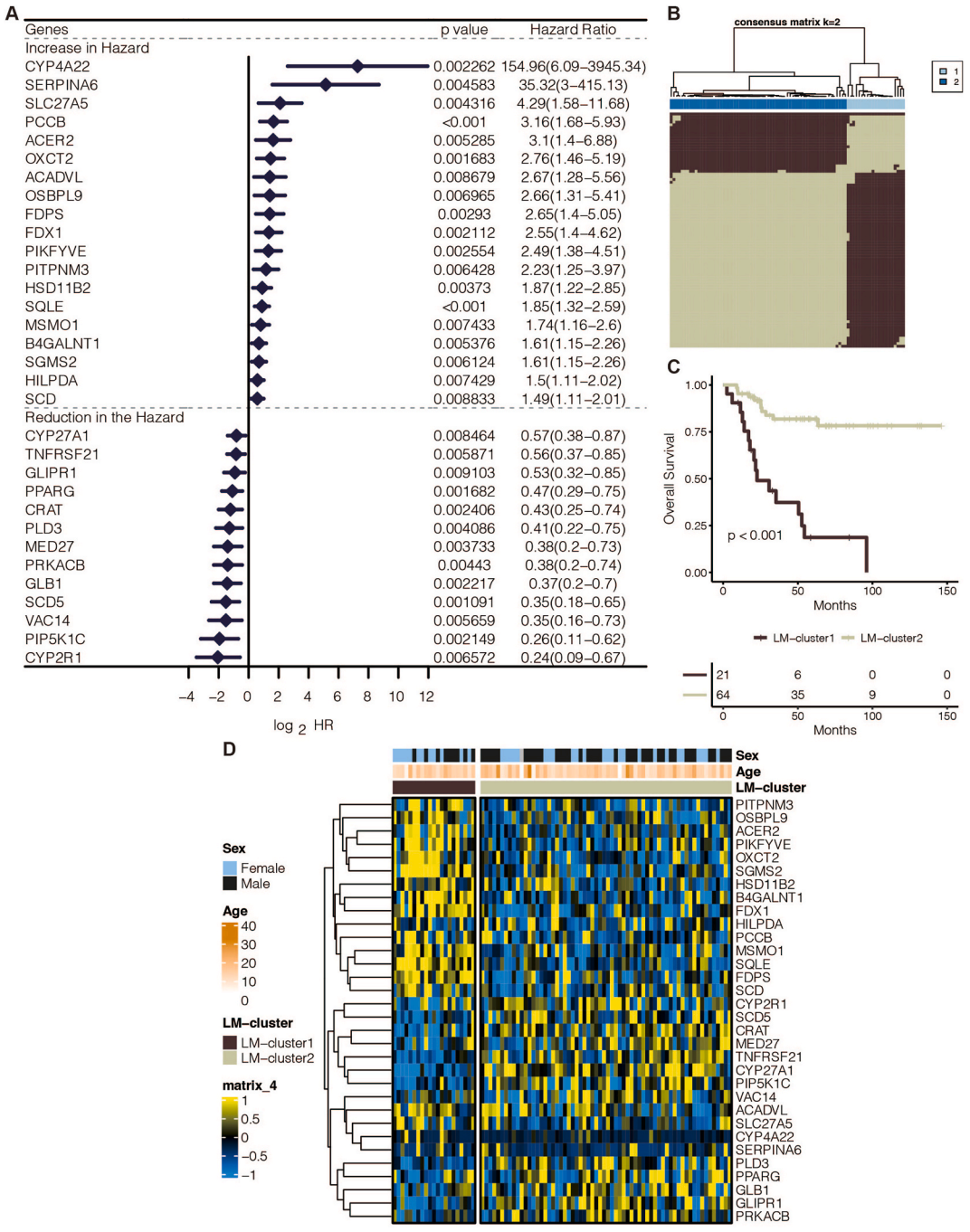

Fig. 1. Identifying the clusters associated with lipid metabolism. A. A univariate Cox regression analysis was performed in the TARGET cohort to identify predictive lipid metabolism-related genes, using a P value cutoff of less than 0.001. B. The partition around medoids (PAM) method was used to generate a consensus matrix with a k value of 2 in the TARGET cohort. C. The survival curve in the TARGET cohort was plotted to compare the survival outcomes between the two clusters with connections to lipid metabolism. D. The heatmap in the TARGET cohort visualizes the expression variations of the prognostic lipid metabolism-related genes between the two lipid metabolism-related clusters.

The analysis used a threshold of a P value less than 0.001 and resulted in the identification of 32 prognostic lipid metabolism-related genes, as shown in Fig. 1A. The consensus matrix, generated using the partition around medoids (PAM) method with a k value of 2, is displayed in Fig. 1B. The survival curve comparing the two lipid metabolism-related clusters in the TARGET cohort revealed that osteosarcoma patients in cluster 1 had significantly decreased survival time, as depicted in Fig. 1C. Additionally, the heatmap in Fig. 1D illustrates the prognostic lipid metabolism-related genes between the two lipid metabolism-related clusters in the TARGET cohort.

2.2. Functions of the two lipid metabolism-related clusters

The volcano plot was used to depict the TARGET cohort's DEGs (Fig. 2B). The box plot displayed the KEGG pathways that were

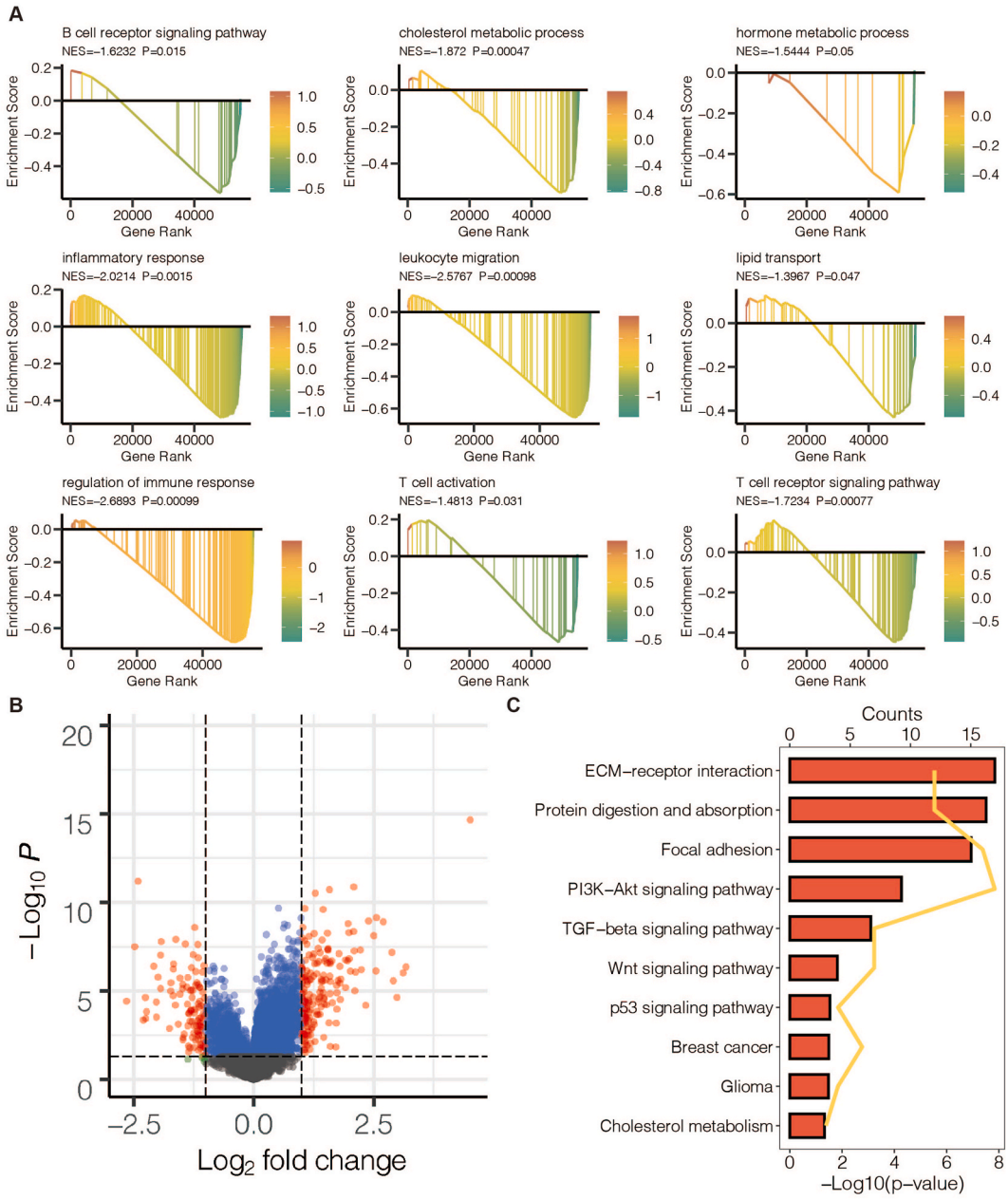

Fig. 2. Annotation of the two clusters linked to lipid metabolism. A. Box plot in the TARGET cohort displayed the KEGG pathways connecting two clusters related to lipid metabolism. B. The DEGs in the TARGET cohort were portrayed using the volcano plot. C. A bar plot in the TARGET cohort highlighted the BP routes between two clusters related to lipid metabolism.

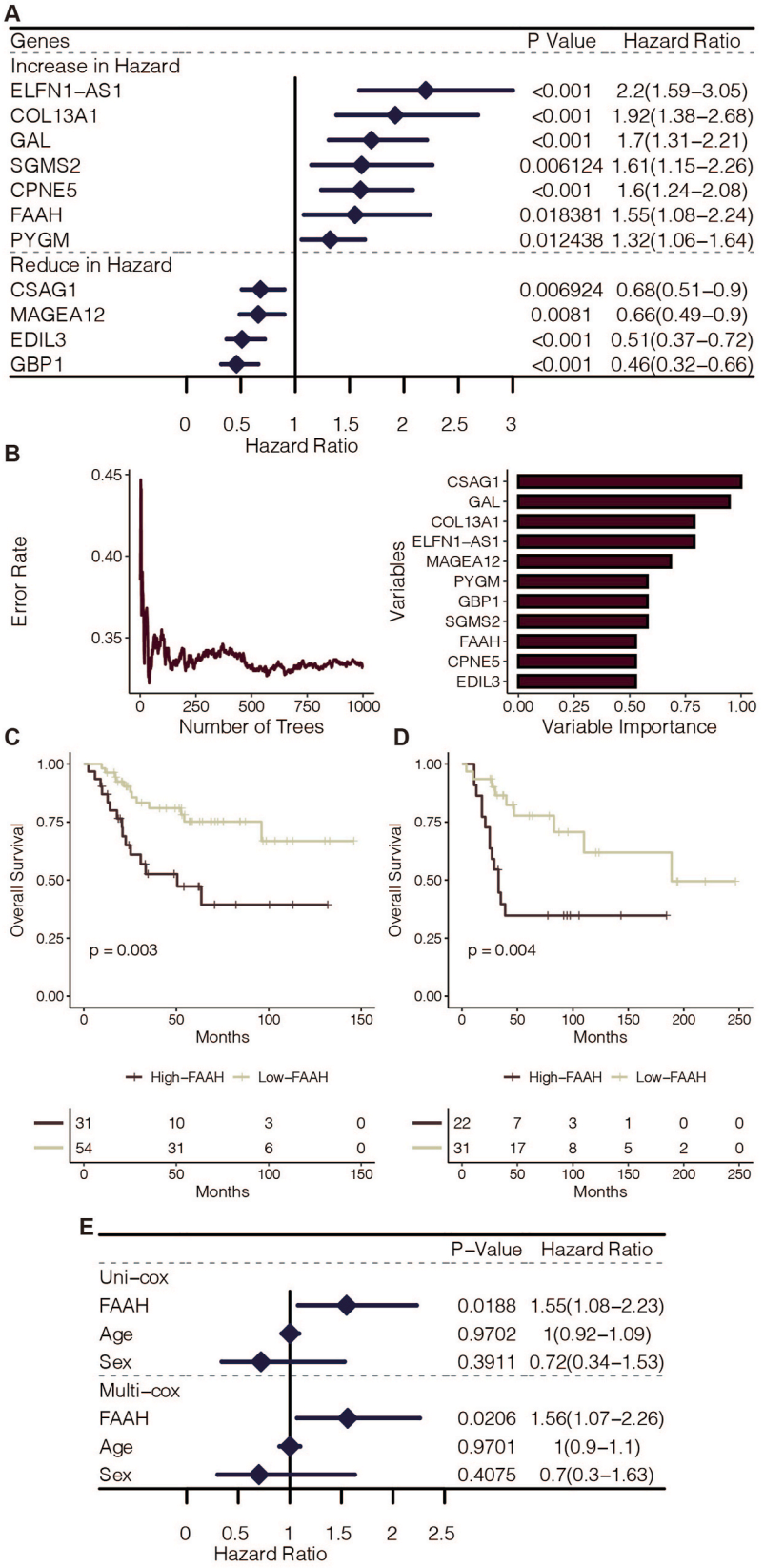

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Fig. 3. Identification of FAAH as a hazardous marker. A univariate Cox regression analysis was used to examine the predicted DEGs in the cohort. B. The Random Survival Forest algorithm's error rate was evaluated in the TARGET cohort. The survival curves between the two FAAH-stratified groups were plotted in both the C. TARGET cohort and the D. GSE21257 cohort. E. Additionally, the TARGET cohort underwent univariate and multivariate Cox regression analyses to identify prognostic markers in addition to FAAH.

different between the two lipid metabolism-associated clusters in the TARGET cohort. It showed that cholesterol metabolic process, inflammatory response, lipid transport, regulation of immune response, T cell activation, and hormone metabolic process were significantly more active in cluster 2 (Fig. 2A). On the other hand, the bar plot demonstrated that the BP pathways, such as the TGF-beta signaling pathway, were highly enriched in terms of the DEGs between the two lipid metabolism-associated clusters in the TARGET cohort (Fig. 2C).

2.3. Identification of FAAH as a hazardous marker

A univariate Cox regression analysis was performed in the TARGET cohort for predictive DEGs, which came to ELFN1-AS1, COL13A1, GAL, SGMS2, CPNE5, FAAH, PYGM, CSAG1, MAGEA12, EDIL3, and GBP1 (Fig. 3A). The error rate of the Random

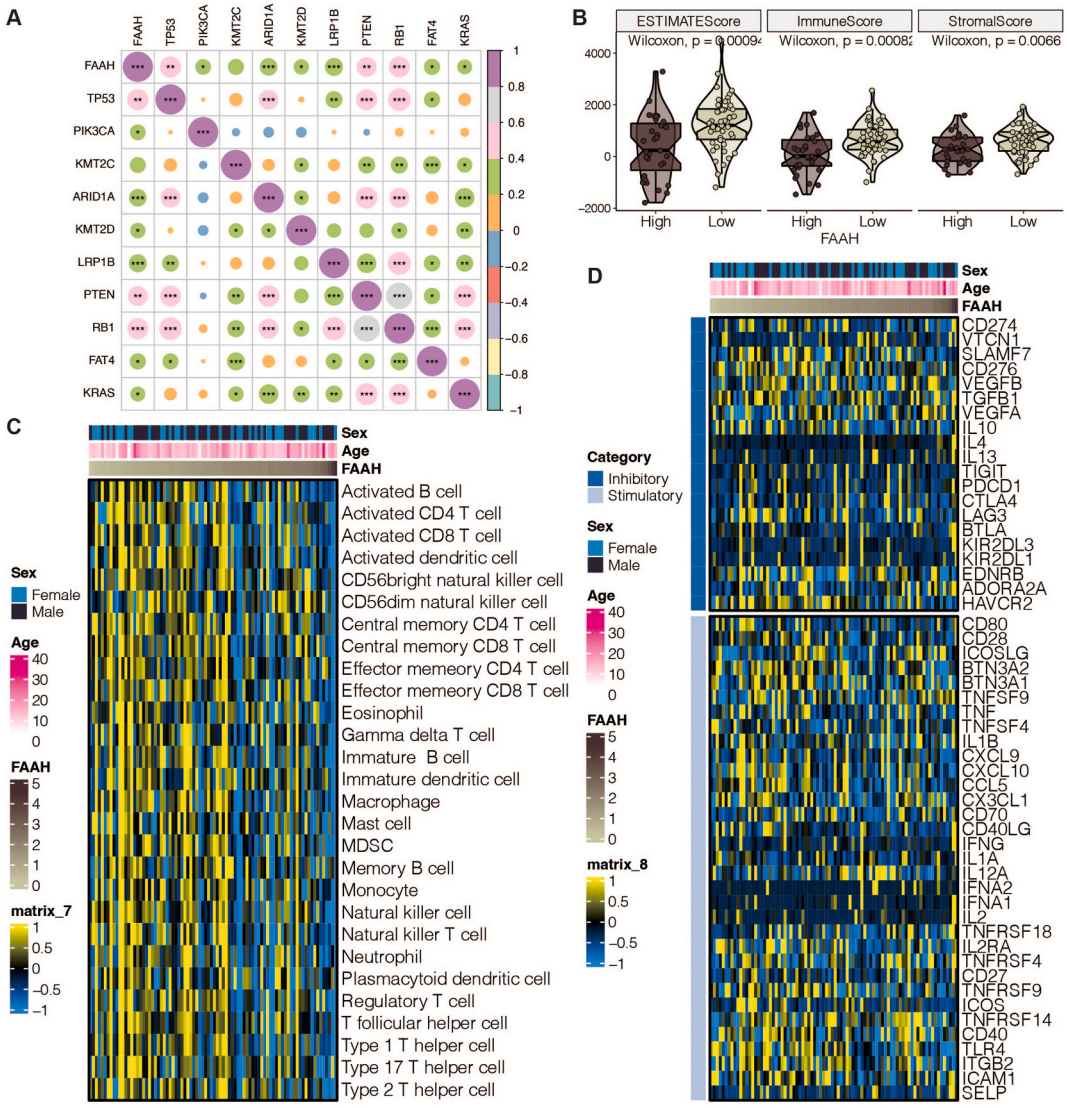

Fig. 4. Oncogenic characteristics and immune evaluation of FAAH. A. Corrplot was used to visualize the relationship between FAAH and oncogenes in the TARGET cohort. B. A box plot was generated to compare the microenvironment scores between the two FAAH-stratified groups in the TARGET cohort. C. A heatmap was created to show the correlation between FAAH expression and immune infiltrating cells in the TARGET cohort. D. Another heatmap was generated to illustrate the relationship between FAAH and immune modulators in the TARGET cohort.

Survival Forest algorithm and the variable importance of predictive DEGs in the TARGET cohort are shown in Fig. 3B. The survival curves between two FAAH-stratified groups in the TARGET cohort (Fig. 3C) and GSE21257 (Fig. 3D) showed that osteosarcoma patients with high FAAH expression had significantly decreased survival time. In order to identify the prognostic factors in addition to FAAH, the univariate Cox regression analysis, and multivariate Cox regression analysis were carried out in the TARGET cohort (Fig. 3E).

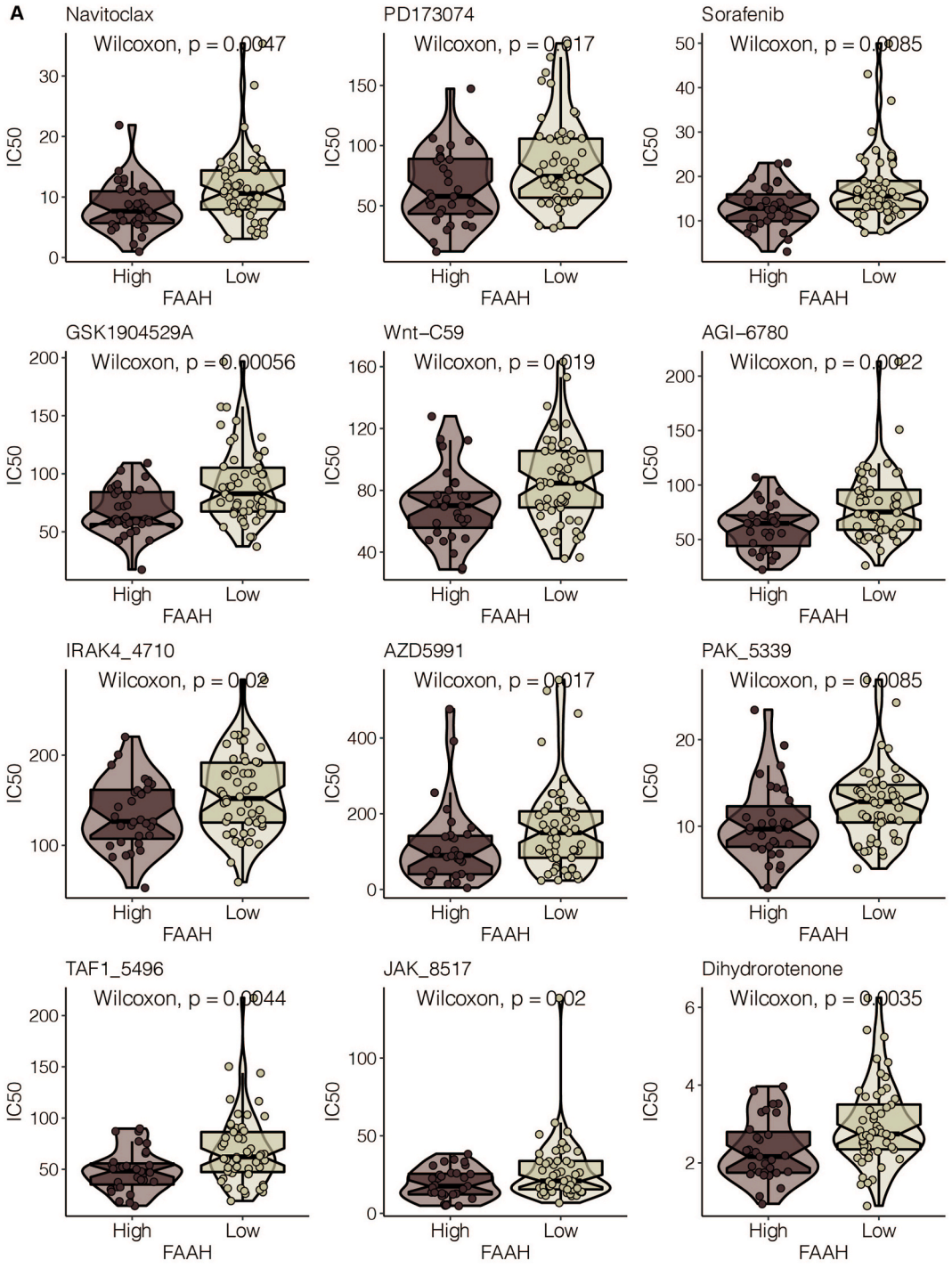

Fig. 5. Drug prediction of FAAH. A. The TARGET cohort's R tool Oncopredict was the foundation for the medication prediction.

2.3.1. Oncogenic and immune analysis of FAAH

Nine common oncogenes, TP53, PIK3CA, ARID1A, KMT2D, LRP1B, PTEN, RB1, FAT4, and KRAS, were significantly associated with FAAH (Fig. 4A). The box plot of microenvironment scores in two FAAH-stratified groups in the TARGET cohort showed that osteosarcoma patients with high FAAH had significantly lower ESTIMATE score, Immune score, and Stromal score (Fig. 4B). In the TARGET cohort, the heatmap demonstrated the relationship between FAAH and immune-invading cells (Fig. 4C). Additionally, the heatmap

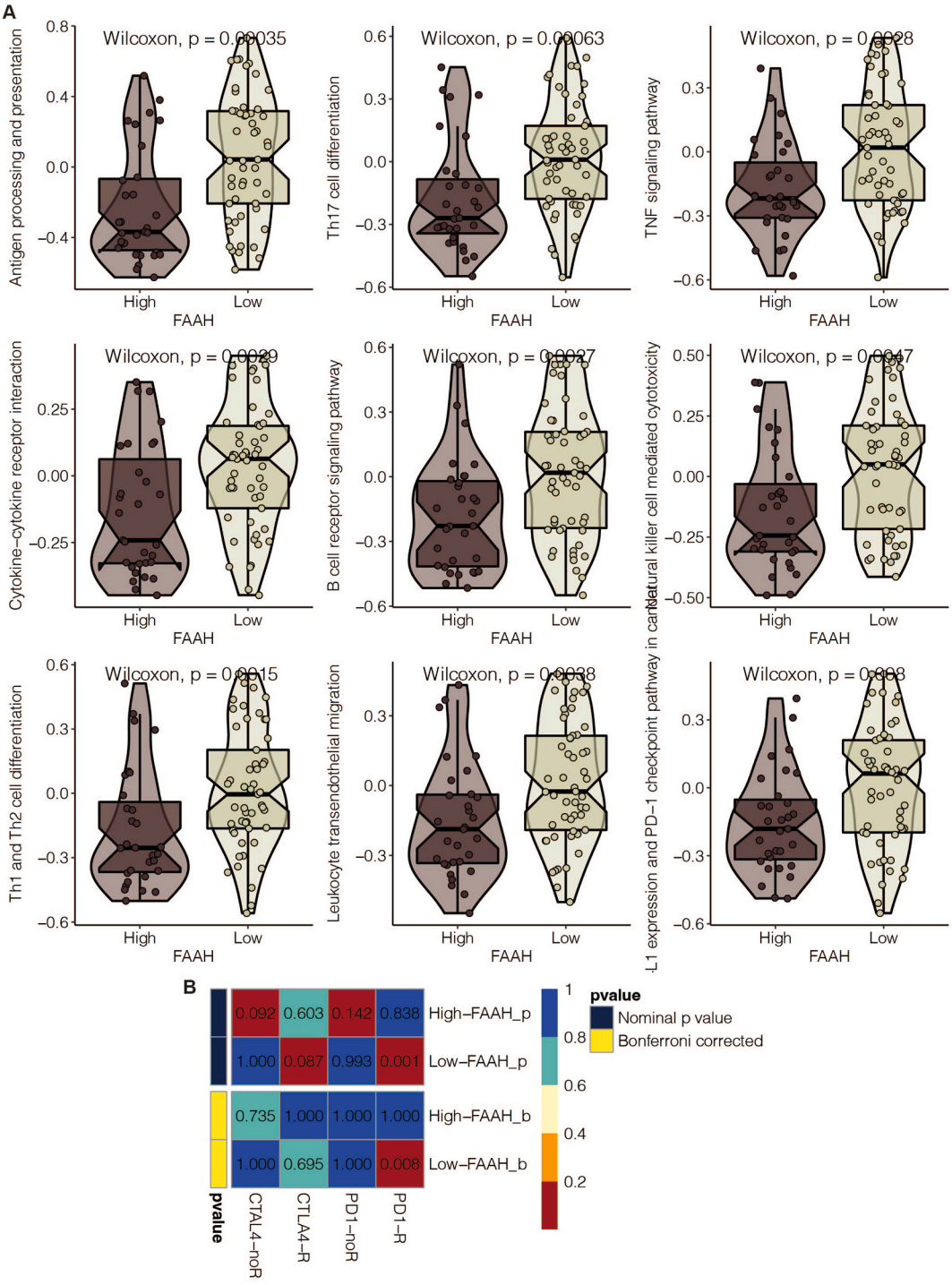

Fig. 6. Immunotherapy prediction of FAAH. A. Box plot in the TARGET cohort displayed the KEGG pathways in two FAAH-stratified groups. B. Submap analysis on FAAH for predicting *anti*-PD1 and *anti*-CTLA4 immunotherapy response.

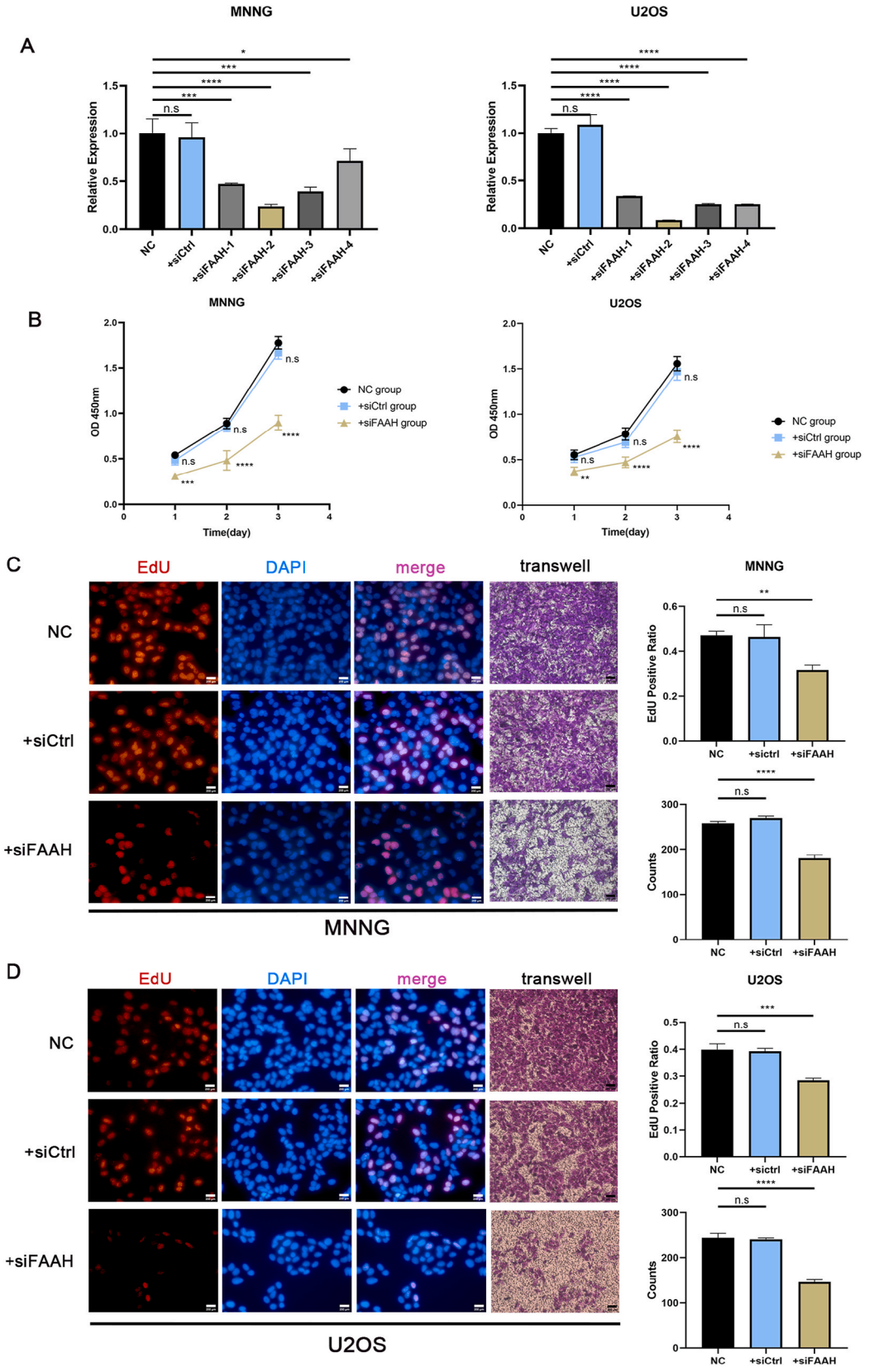

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Fig. 7. Experimental validation was conducted on FAAH, as described in the following experiments: A. The expression of FAAH was assessed in two cell lines (MNNG and U2OS) across five groups using RT-qPCR. B. Cell proliferation was measured in MNNG and U2OS knockdown cells using the CCK-8 assay. C. The EdU assay was employed to investigate cell proliferation in the MNNG/HOS cell line among three groups. D. The EdU assay was employed to investigate cell proliferation in the U2OS cell line among three groups. In these experiments, "si-FAAH" denotes the transfection of FAAH-specific siRNA, while "si-NC" represents nonspecific control siRNA transfection. The statistical significance levels are indicated as follows: ns (no significance), $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

displayed the relationship between FAAH and immune modulators in the TARGET cohort (Fig. 4D).

2.3.2. Drug prediction of FAAH

The drug prediction was based on the R package Oncopredict in the TARGET cohort, which Navitoclax, PD173074, Sorafenib, GSK1904529A, Wnt-C59, AGI-6780, IRAK4_4710, AZD5991, PAK_5339, TAF1_5496, JAK_8517, and Dihydrorotenone had significantly lower IC50 in osteosarcoma patients with high FAAH (Fig. 5A). The molecular docking between FAAH and predicted drugs showed that the binding energy between FAAH and AZD5991, Dihydrorotenone, PD173074, Sorafenib, and Wnt-C59 were −8.586, −9.353, −8.308, −9.142, and −10.793, respectively (Fig. S1).

2.3.3. Immunotherapy prediction of FAAH

The GSVA of KEGG pathways was applied to the DEGs between two FAAH-related groups, in which oncogenic and immune pathways in cancer were significantly more activated in osteosarcoma patients with low FAAH (Fig. 6A). Submap analysis showed that osteosarcoma patients with low FAAH had significantly positive responsiveness of immunotherapy against PD-1 (Fig. 6B). Additionally, in four cohorts (Kim, Wolf, Hugo, and Homet), FAAH was reliable in screening out immunotherapy beneficiaries, with AUC values of 0.75, 0.736, 0.723, and 0.767, respectively (Fig. S2A). However, in contrast to the Kim cohort, which had better survival outcomes, FAAH was related to shortened survival time in the Hugo and IMvigor210 cohorts (Fig. S2B).

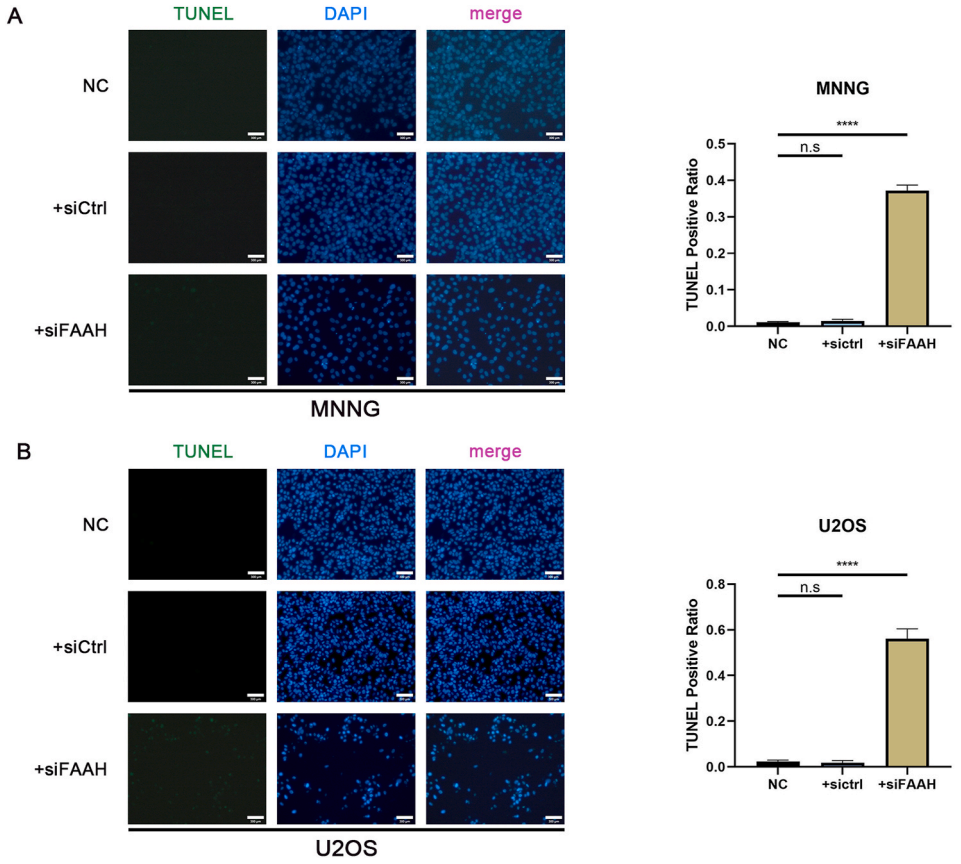

Fig. 8. Experimental validation was conducted on FAAH, as described in the following experiments: A. The TUNEL assay was employed to investigate cell apoptosis in the MNNG/HOS cell line among three groups. B. The TUNEL assay was employed to investigate cell apoptosis in the U2OS cell line among three groups. The statistical significance levels are indicated as follows: ns (no significance), $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

2.3.4. In vitro validation of FAAH

Given the potent prognostic value of FAAH, in vitro validation was further performed to confirm its oncogenic role. RT-qPCR was employed to assess FAAH expression across five groups (NC, si-NC, si-FAAH-1, si-FAAH-2, si-FAAH-3, si-FAAH-4) in two distinct cell lines to determine the most potent siRNA (Fig. 7A). Using the CCK-8 assay, cell proliferation was quantified in MNNG/HOS and U2OS knockdown cells, in which osteosarcoma cells in the si-FAAH group had significantly decreased OD values (Fig. 7B). In the MNNG/HOS cell line, the EdU assay was utilized to explore cell proliferation within three groups (NC, si-NC, si-FAAH), in which osteosarcoma cells in the si-FAAH group had significantly decreased proliferation rates (Fig. 7C). In the U2OS cell line, the EdU assay was utilized to explore cell proliferation within three groups (NC, si-NC, si-FAAH), in which osteosarcoma cells in the si-FAAH group had significantly decreased proliferation rates (Fig. 7D). The TUNEL assay was employed to investigate cell apoptosis in the MNNG/HOS cell line, in which osteosarcoma cells in the si-FAAH group had significantly increased apoptotic rates (Fig. 8A). The TUNEL assay was employed to investigate cell apoptosis in the U2OS cell line, in which osteosarcoma cells in the si-FAAH group had significantly increased apoptotic rates (Fig. 8B). In addition, the IHC staining of FAAH in clinical osteosarcoma samples revealed that FAAH was more expressed in tumor tissues than in normal tissues (Fig. 9A).

3. Discussion

The dysregulation of lipid metabolism in cancer supports tumor growth, survival, and metastasis. Targeting key enzymes and pathways involved in lipid metabolism has emerged as a potential therapeutic strategy for cancer treatment [25]. One key aspect of lipid metabolism in cancer is the increased de novo lipogenesis, which is the synthesis of new lipids from non-lipid precursors. Cancer cells often upregulate enzymes involved in this process, such as fatty acid synthase (FASN), to support their rapid growth and proliferation. This increased lipogenesis provides cancer cells with the necessary building blocks for membrane synthesis, energy production, and signaling molecules. Additionally, cancer cells exhibit alterations in lipid uptake and storage [26]. They can increase the uptake of exogenous lipids, such as fatty acids, from the surrounding environment to meet their high energy demands. The upregulation of lipid transporters, such as CD36, can facilitate this. Cancer cells also have an enhanced ability to store lipids in lipid droplets,

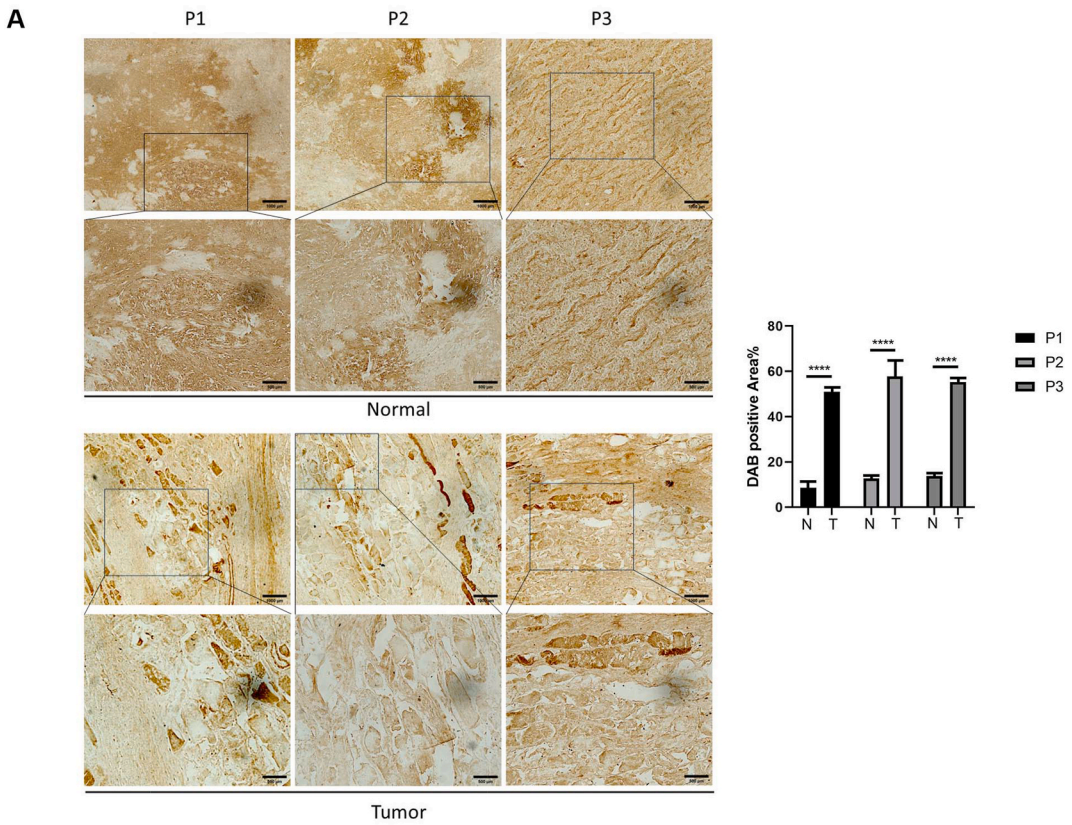

Fig. 9. Experimental validation was conducted on FAAH, as described in the following experiment: A. The expression of FAAH in osteosarcoma tumor tissues and normal tissues was validated in vitro using immunohistochemistry (IHC). The images of the sections were captured at 10× magnification in the upper row and 20× magnification in the lower row. Here, "si-FAAH" denotes the transfection of FAAH-specific siRNA, while "si-NC" represents nonspecific control siRNA transfection. The statistical significance levels are indicated as follows: ns (no significance), $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

which serve as energy and membrane synthesis reservoirs. Furthermore, cancer cells can remodel their lipid metabolism to support survival under nutrient-deprived conditions. They can switch to alternative lipid sources, such as ketone bodies, to sustain their energy needs when glucose availability is limited. This metabolic adaptation allows cancer cells to thrive in nutrient-poor environments, such as solid tumors [27]. In accordance with the previous findings, oncogenic pathways such as the TGF-beta signaling pathway were highly enriched with regard to the DEGs between two lipid metabolism-associated clusters.

In our study, cholesterol metabolic process, inflammatory response, lipid transport, regulation of immune response, T cell activation, and hormone metabolic process were also significantly more active in lipid metabolism-related cluster 2. Lipid metabolism and immune activity are actually closely interconnected [28]. Lipids, which include fats and cholesterol, play important roles in the immune system. Lipids serve as energy sources for immune cells and are involved in producing signaling molecules called cytokines, which regulate immune responses. One key aspect of lipid metabolism in immune activity is the production of eicosanoids [29]. Eicosanoids are lipid-derived signaling molecules that have diverse effects on the immune system. For example, some eicosanoids can promote inflammation, while others can have anti-inflammatory effects. These molecules are produced from certain lipids, such as arachidonic acid, derived from dietary fats. Lipids also form cell membranes, which are crucial for immune cell function [30]. Immune cells, such as macrophages and lymphocytes, have specialized lipid rafts in their membranes that help organize signaling molecules and receptors involved in immune responses. Furthermore, lipid metabolism can influence the development and function of immune cells. For instance, certain lipids, such as omega-3 fatty acids found in fish oil, have been shown to have anti-inflammatory effects and can modulate the activity of immune cells [31]. On the other hand, dysregulation of lipid metabolism can contribute to immune dysfunction and the development of inflammatory diseases.

Our study further identified FAAH as a hazardous marker in osteosarcoma. FAAH is an enzyme that plays a crucial role in the endocannabinoid system [32]. This system regulates various physiological processes, including pain sensation, mood, appetite, and inflammation. FAAH specifically breaks down anandamide, an endocannabinoid that binds to cannabinoid receptors in the brain and body [33]. Understanding the function and regulation of FAAH is important for developing therapeutic strategies for various conditions, such as chronic pain, anxiety, and inflammation. miR-1275 coordinately regulates AEA/LPA signals in gastric cancer by targeting FAAH and altering lipid metabolism [34]. By inhibiting the EGF/EGFR pathway, FAAH inhibition increases anandamide's anti-tumorigenic actions in non-small cell lung cancer [35]. Through the PI3K-AKT signaling pathway, therapy with FAAH inhibitors/URB597 and ferroptosis inducers greatly reduce the development and spread of renal cell carcinoma cells [36]. Recent studies have also suggested a potential link between FAAH and osteosarcoma, indicating that targeting FAAH may have therapeutic implications for treating this cancer [37]. The in vitro validation also proved that FAAH could facilitate growth and migration and inhibit the apoptosis of osteosarcoma. Besides, FAAH is more expressed in tumor tissues than in normal tissues.

The microenvironment scores were significantly lower in osteosarcoma patients with high FAAH. FAAH was also negatively associated with immune cells, indicating an immune cold microenvironment in osteosarcoma patients with high FAAH. Besides, oncogenic and immune pathways in cancer were significantly more activated in osteosarcoma patients with low FAAH. Studies have shown that the endocannabinoid system, including FAAH, modulates immune activity [38]. Anandamide, which is a substrate for FAAH, has been shown to have an impact on the production of pro-inflammatory cytokines. Cytokines are signaling molecules that play a crucial role in immune responses, and their production can be influenced by various factors, including anandamide. The endocannabinoid system has also regulated immune cell migration and function.

In addition, FAAH was negatively associated with immune modulators, such as TGF-beta, LAG3, HAVCR2, CD40, CD274, and CD276. Osteosarcoma patients with low FAAH had significantly positive responsiveness to immunotherapy against PD-1. FAAH demonstrated high sensitivity in predicting immunotherapy beneficiaries in four immunotherapy cohorts and could stratify patients' survival in three immunotherapy cohorts. According to the previous literature, FAAH is not directly connected to cancer immunotherapy. However, it has been found that FAAH inhibitors can enhance the anti-tumor immune response in certain cancer models. Inhibition of FAAH can increase the levels of endocannabinoids, which are lipid signaling molecules that can modulate immune responses [39]. These endocannabinoids can activate cannabinoid receptors on immune cells, suppressing tumor growth and enhancing anti-tumor immune responses [40]. So, while FAAH may not be directly involved in cancer immunotherapy, its inhibition can have potential therapeutic implications.

The drug prediction of FAAH indicated that Navitoclax, PD173074, Sorafenib, GSK1904529A, Wnt-C59, AGI-6780, IRAK4_4710, AZD5991, PAK_5339, TAF1_5496, JAK_8517, and Dihydrorotenone had significantly lower IC50 in osteosarcoma patients with high FAAH. Navitoclax specifically targets B-cell lymphoma 2 (BCL-2) proteins, which regulate cell survival. PD173074 is a selective inhibitor of fibroblast growth factor receptor 1 (FGFR1). Sorafenib is a multi-kinase inhibitor that targets several signaling pathways in tumor growth and angiogenesis. GSK1904529A is a selective inhibitor of insulin-like growth factor 1 receptor (IGF-1R) involved in cell growth and survival. Wnt-C59 is a small molecule inhibitor that targets the Wnt signaling pathway. AGI-6780 is a selective inhibitor of the bromodomain and extra-terminal (BET) family of proteins involved in gene regulation. IRAK4_4710 is a small molecule inhibitor that targets interleukin-1 receptor-associated kinase 4 (IRAK4), which is involved in the immune response. It has been studied as a potential treatment for various types of cancer, including lymphoma and leukemia. AZD5991 is a selective inhibitor of MCL-1, a protein involved in cell survival. PAK_5339 is a small molecule inhibitor that targets p21-activated kinase (PAK), which is involved in cell proliferation and migration. TAF1_5496 is a small molecule inhibitor that targets TATA-binding protein-associated factor 1 (TAF1), which regulates gene regulation. JAK_8517 is a small molecule inhibitor that targets Janus kinase (JAK), which is involved in cell signaling. Dihydrorotenone is a natural compound that has been studied for its potential anticancer properties. The molecular docking showed that FAAH could bind to AZD5991, Dihydrorotenone, PD173074, Sorafenib, and Wnt-C59 with high binding energy.

In sum, FAAH was discovered to be a powerfully dangerous gene in osteosarcoma. Future research will focus on how FAAH may alter the immune and drug responses to osteosarcoma.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

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CRediT authorship contribution statement

Yanbin Shi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Song Wu:** Supervision, Resources, Project administration, Funding acquisition. **Xiaolin Zhang:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation. **Yangbo Cao:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Lina Zhang:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23499.

References

[1] B.A. Lindsey, J.E. Markel, E.S. Kleinerman, Osteosarcoma overview, Rheumatol Ther 4 (2017) 25–43.
[2] S.J. Strauss, A.M. Frezza, N. Abecassis, J. Bajpai, S. Bauer, R. Biagini, et al., Bone sarcomas: ESMO-EURACAN-GENTURIS-ERN PaedCan clinical practice guideline for diagnosis, treatment and follow-up, Ann. Oncol. 32 (2021) 1520–1536.
[3] S. Cole, D.M. Gianferante, B. Zhu, L. Mirabello, Osteosarcoma: a surveillance, epidemiology, and end results program-based analysis from 1975 to 2017, Cancer 128 (2022) 2107–2118.
[4] T. Heare, M.A. Hensley, S. Dell'Orfano, Bone tumors: osteosarcoma and Ewing's sarcoma, Curr. Opin. Pediatr. 21 (2009) 365–372.
[5] J.W. Martin, J.A. Squire, M. Zielenska, The genetics of osteosarcoma, Sarcoma 2012 (2012), 627254.
[6] C. Cheng, F. Geng, X. Cheng, D. Guo, Lipid metabolism reprogramming and its potential targets in cancer, Cancer Commun. 38 (2018) 27.
[7] Z. Ren, S. Wang, B. Li, H. Huang, H. Zhang, Z. Yang, et al., Hsa_circ_0000073 promotes lipid synthesis of osteosarcoma through hsa-miR-1184/FADS2 pathway, Cell. Signal. 110 (2023), 110829.
[8] W. Tong, S. Wang, C. He, A. Li, J. Nie, W. Zuo, et al., CircREOS suppresses lipid synthesis and osteosarcoma progression through inhibiting HuR-mediated MYC activation, J. Cancer 14 (2023) 916–926.
[9] X. Ding, Y. Zhang, J. Liang, Q. Li, H. Hu, Y. Zhou, et al., Dihydroartemisinin potentiates VEGFR-TKIs antitumorigenic effect on osteosarcoma by regulating Loxl2/VEGFA expression and lipid metabolism pathway, J. Cancer 14 (2023) 809–820.
[10] Y. Zhou, D. Yang, Q. Yang, X. Lv, W. Huang, Z. Zhou, et al., Single-cell RNA landscape of intratumoral heterogeneity and immunosuppressive microenvironment in advanced osteosarcoma, Nat. Commun. 11 (2020) 6322.
[11] C. Chen, L. Xie, T. Ren, Y. Huang, J. Xu, W. Guo, Immunotherapy for osteosarcoma: fundamental mechanism, rationale, and recent breakthroughs, Cancer Lett. 500 (2021) 1–10.
[12] N. van Egmond, V.M. Straub, M. van der Stelt, Targeting endocannabinoid signaling: FAAH and MAG lipase inhibitors, Annu. Rev. Pharmacol. Toxicol. 61 (2021) 441–463.
[13] J.E. Schlosburg, S.G. Kinsey, A.H. Lichtman, Targeting fatty acid amide hydrolase (FAAH) to treat pain and inflammation, AAPS J. 11 (2009) 39–44.
[14] O. Gunduz-Cinar, M.N. Hill, B.S. McEwen, A. Holmes, Amygdala FAAH and anandamide: mediating protection and recovery from stress, Trends Pharmacol. Sci. 34 (2013) 637–644.
[15] H. Deng, W. Li, Monoacylglycerol lipase inhibitors: modulators for lipid metabolism in cancer malignancy, neurological and metabolic disorders, Acta Pharm. Sin. B 10 (2020) 582–602.
[16] R. Ramer, F. Wittig, B. Hinz, The endocannabinoid system as a pharmacological target for new cancer therapies, Cancers 13 (2021).
[17] L. Liu, M. Mo, X. Chen, D. Chao, Y. Zhang, X. Chen, et al., Targeting inhibition of prognosis-related lipid metabolism genes including CYP19A1 enhances immunotherapeutic response in colon cancer, J. Exp. Clin. Cancer Res. 42 (2023) 85.
[18] F. Martinez-Jimenez, F. Muinos, I. Sentis, J. Deu-Pons, I. Reyes-Salazar, C. Arnedo-Pac, et al., A compendium of mutational cancer driver genes, Nat. Rev. Cancer 20 (2020) 555–572.
[19] P. Charoentong, F. Finotello, M. Angelova, C. Mayer, M. Efremova, D. Rieder, et al., Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade, Cell Rep. 18 (2017) 248–262.
[20] K. Yoshihara, M. Shahmoradgoli, E. Martinez, R. Vegesna, H. Kim, W. Torres-Garcia, et al., Inferring tumour purity and stromal and immune cell admixture from expression data, Nat. Commun. 4 (2013) 2612.
[21] D. Maeser, R.F. Gruener, R.S. Huang, oncoPredict: an R package for predicting in vivo or cancer patient drug response and biomarkers from cell line screening data, Briefings Bioinf. (2021) 22.
[22] G.M. Morris, R. Huey, A.J. Olson, Using AutoDock for ligand-receptor docking, Curr Protoc Bioinformatics (2008) (Chapter 8): Unit 8 14.
[23] Y. Wang, S.H. Bryant, T. Cheng, J. Wang, A. Gindulyte, B.A. Shoemaker, et al., PubChem BioAssay: 2017 update, Nucleic Acids Res. 45 (2017) D955–D963.

[24] Y. Hoshida, J.P. Brunet, P. Tamayo, T.R. Golub, J.P. Mesirov, Subclass mapping: identifying common subtypes in independent disease data sets, PLoS One 2 (2007) e1195.
[25] X. Bian, R. Liu, Y. Meng, D. Xing, D. Xu, Z. Lu, Lipid metabolism and cancer, J. Exp. Med. (2021) 218.
[26] L.A. Broadfield, A.A. Pane, A. Talebi, J.V. Swinnen, S.M. Fendt, Lipid metabolism in cancer: new perspectives and emerging mechanisms, Dev. Cell 56 (2021) 1363–1393.
[27] D.W. Jeong, S. Lee, Y.S. Chun, How cancer cells remodel lipid metabolism: strategies targeting transcription factors, Lipids Health Dis. 20 (2021) 163.
[28] W. Yu, Q. Lei, L. Yang, G. Qin, S. Liu, D. Wang, et al., Contradictory roles of lipid metabolism in immune response within the tumor microenvironment, J. Hematol. Oncol. 14 (2021) 187.
[29] M.M. Ching, J. Reader, A.M. Fulton, Eicosanoids in cancer: prostaglandin E(2) receptor 4 in cancer therapeutics and immunotherapy, Front. Pharmacol. 11 (2020) 819.
[30] R.F. Saito, L.N.S. Andrade, S.O. Bustos, R. Chammas, Phosphatidylcholine-derived lipid mediators: the crosstalk between cancer cells and immune cells, Front. Immunol. 13 (2022), 768606.
[31] Y. Fu, Y. Wang, H. Gao, D. Li, R. Jiang, L. Ge, et al., Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity, Mediat. Inflamm. 2021 (2021), 8879227.
[32] D.G. Deutsch, N. Ueda, S. Yamamoto, The fatty acid amide hydrolase (FAAH), Prostaglandins Leukot. Essent. Fatty Acids 66 (2002) 201–210.
[33] E. Dainese, S. Oddi, M. Simonetti, A. Sabatucci, C.B. Angelucci, A. Ballone, et al., The endocannabinoid hydrolase FAAH is an allosteric enzyme, Sci. Rep. 10 (2020) 2292.
[34] Q. Yang, S. Kong, J. Yu, Y. Xu, M. Tao, S. Ma, et al., MicroRNA miR-1275 coordinately regulates AEA/LPA signals via targeting FAAH in lipid metabolism reprogramming of gastric cancer, Cell Death Dis. 14 (2023) 62.
[35] J. Ravi, A. Sneh, K. Shilo, M.W. Nasser, R.K. Ganju, FAAH inhibition enhances anandamide mediated anti-tumorigenic effects in non-small cell lung cancer by downregulating the EGF/EGFR pathway, Oncotarget 5 (2014) 2475–2486.
[36] J. Hao, Q. Chen, Y. Feng, Q. Jiang, H. Sun, B. Deng, et al., Combination treatment with FAAH inhibitors/URB597 and ferroptosis inducers significantly decreases the growth and metastasis of renal cell carcinoma cells via the PI3K-AKT signaling pathway, Cell Death Dis. 14 (2023) 247.
[37] J. Roy, J.E. Watson, I.S. Hong, T.M. Fan, A. Das, Antitumorigenic properties of omega-3 endocannabinoid epoxides, J. Med. Chem. 61 (2018) 5569–5579.
[38] M. Kienzl, J. Kargl, R. Schicho, The immune endocannabinoid system of the tumor microenvironment, Int. J. Mol. Sci. 21 (2020).
[39] M. Grieco, M.G. De Caris, E. Maggi, F. Armeli, R. Coccurello, T. Bisogno, et al., Fatty acid amide hydrolase (FAAH) inhibition modulates amyloid-beta-induced microglia polarization, Int. J. Mol. Sci. 22 (2021).
[40] O. Almogi-Hazan, R. Or, Cannabis, the endocannabinoid system and immunity-the journey from the bedside to the bench and back, Int. J. Mol. Sci. 21 (2020).