Dear Editor,

Apolipoprotein C2 (APOC2), an activator of lipoprotein lipase, participates in hydrolysis of triglycerides, very low-density lipoproteins, and high-density lipoproteins to release free fatty acids (FA).\(^1\) FA oxidation has emerged as an important source of energy for cancer survival and growth.\(^2\) Patterns of APOC2 genomics and transcriptomic alterations in cancer remained unexplored. Here, we characterize APOC2 deregulation in cancer by analyzing 176 studies (supplementary-methods).

In 46706 samples of 34 different cancers (Table S1), amplification, mutation, and deep deletion were the main identified APOC2 alterations (Figure S1A). Approximately 1% (251 patients) had at least one genetic alteration in APOC2 with the highest frequency (9.72%) present in bladder cancer and gene amplification being the most common alteration. The highest frequency of deep deletions in APOC2 occurred in diffuse glioma (1.36%). APOC2 mutations occurred in 2.3% of non-melanoma skin cancer and at lower frequencies in other cancers (Table S2). Among the 26 different APOC2 missense and truncating mutations, two were previously reported in hypertriglyceridemia (Figure S1B; Tables S3 and S4).

APOC2 was expressed at significantly higher levels in several malignancies (Figures 1 and S2), such as patient-derived glioblastoma stem cells (33.7-fold, \(p < 0.0001\); Figure 1A), invasive ductal breast cancer (2.9-fold, \(p < 0.0001\); Figure 1B), centroblastic lymphoma (10.1-fold, \(p < 0.0001\); Figure 1C), early stage colorectal tumor (3.8-fold, \(p = 0.0001\); Figure 1D), hypopharyngeal cancer (2.1-fold, \(p = 0.04\); Figure 1E), clear cell renal cell carcinoma (5.9-fold, \(p < 0.001\); Figure 1F), skin squamous cell carcinoma (2.3-fold, \(p = 0.023\); Figure 1G), and gastric cancer (4.8-fold, \(p = 0.002\); Figure 1H) compared with the corresponding normal tissues.

We also compared APOC2 DNA methylation beta (\(\beta\)) values corresponding to APOC2 gene between primary tumors and normal tissues. APOC2 median \(\beta\)-values were significantly lower in tumor samples compared with controls (breast invasive carcinoma: 0.809 vs. 0.852; \(p < 0.0001\); Figure 2A; colon adenocarcinoma: 0.7 vs. 0.81; \(p < 0.0001\); Figure 2B; kidney renal clear cell carcinoma: 0.79 vs. 0.83; \(p < 0.0001\); Figure 2C; and stomach adenocarcinoma: 0.676 vs. 0.772; \(p = 0.116\); Figure 2D).

The association between APOC2 alteration and chromosomal alterations, clinical attributes, and mutational status are summarized in Figure S3 and Table S5. APOC2 gene alterations were more frequently found in patients with TP53, RYRI, RYR2, and LRPIB mutations (\(p < 0.01\); Table S6).

We further evaluated cell signaling pathways associated with APOC2 genetic alterations (amplification, mutation, and high mRNA levels) by ingenuity pathway analysis in several solid malignancies. Several pathways involved in lipid metabolism were altered (the upregulation of phospholipase C signaling and the downregulation of PPAR signaling), and pathways involved in innate and adaptive immune response were also modulated (Figure S4). The dendritic cells maturation pathway, pathways involved in the cross talk between dendritic cells and natural killer cells, Th1 and Th2 activation pathway were upregulated, while the PD-1 and PD-L1 cancer immunotherapy pathway was downregulated (Tables S8 and S9).

Survival analysis on all patients from different datasets showed that patients with APOC2 alterations (mutations and copy number alterations) had significantly shorter median overall survival (OS) compared with patients without alterations (OS: 37.97 vs. 80.68 months; \(p < 0.0001\); Figure 3A). Association between altered APOC2 and poor OS was also observed in several types of cancers (Table S10). In esophageal adenocarcinoma, patients with high APOC2 expression exhibited shorter OS (13.48 vs. 28.11 months; \(p = 0.0363\); Figure 3B). In stomach adenocarcinoma, patients with high APOC2 had a significantly shorter OS (14.96 vs. 36.00 months; \(p = 0.0072\); Figure 3C) than...
FIGURE 1 Analysis of APOC2 expression in different cancers. APOC2 expression data were retrieved from the GEO database. The mean fold change was compared between cancer group and healthy control in (A) patient-derived glioma stem cells, (B) invasive ductal breast carcinoma, (C) centroblastic lymphoma, (D) early stage colorectal tumor, (E) hypopharyngeal cancer, (F) clear cell renal cell carcinoma, (G) skin squamous cell carcinoma, and (H) gastric cancer. The differences between groups were analyzed by unpaired t-test, with the exception of panel (B), (D), and (E), which were analyzed by Mann-Whitney test. (** p < 0.01, *** p < 0.001, **** p < 0.0001).

Patients with low APOC2. In ovarian serous cystadenocarcinoma, patients with APOC2 mutation or high expression had significantly poorer OS than patients with wild-type APOC2 and low gene expression (35.80 vs. 44.51 months; p = 0.0058; Figure 3D).

Metabolic reprogramming is a hallmark of cancer cells to support tumorigenesis and tumor progression. Lipid metabolism is elevated in cancer to satisfy increased energy demands. Considering APOC2’s known function in FA transport and metabolism, its upregulation is a plausible mechanism for cancer adaptation and growth. By analyzing public genomics data, we found that APOC2 was upregulated and hypomethylated in several types of cancers. We recently reported APOC2 upregulation and hypomethylation in acute myeloid leukemia (AML) and more notably in AML with mixed-lineage leukemia (MLL)-rearrangements suggesting an epigenetic mechanism regulating APOC2 expression. Additionally, the transcription factor STAT1 interacts with RXRα on APOC2 promoter to drive APOC2 expression in macrophages. Upregulation of APOC2 in gastrointestinal stromal tumor was associated with cancer cell proliferation, migration, and invasion. Targeting APOC2 in AML caused antileukemia activity via inhibiting CD36-ERK pathway. Consistent with our findings, high APOC2 serum levels were previously found to be associated with shorter survival in pancreatic cancer. Upregulation of APOC2 was also found in metastatic tumors such as breast invasive carcinoma and fast-growing lymphoma, suggesting that APOC2 may serve as a prognostic biomarker in cancer. APOC2 deregulations are associated with TP53 mutations. TP53 mutations are enriched in early clonal stages regardless of tumor type. Whether APOC2 upregulation is also an early event in tumor evolution remains to be
FIGURE 2 DNA methylation levels of APOC2 in cancers where APOC2 is upregulated. Analysis was performed for (A) breast invasive carcinoma, (B) colon adenocarcinoma, (C) kidney renal clear cell carcinoma, and (D) stomach adenocarcinoma. The differences between groups were analyzed by t-test. (** p < 0.0001). Abbreviation: ns, not significant

APOC2 gene alterations and upregulation occur frequently in cancer. Functional and mechanistic studies establishing APOC2 as a therapeutic target and expanding these analyses to other apolipoprotein family members are warranted.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
FIGURE 3  Survival analysis for patients with different cancers associated with APOC2 alteration and mRNA expression. (A) Overall survival of cancer patients with and without APOC2 alterations including APOC2 mutation as well as copy number alterations. (B) Overall survival of esophageal adenocarcinoma patients with high (Z ≥ 2) and low (Z < 2) APOC2 mRNA level. (C) Overall survival of stomach adenocarcinoma patients with high (Z ≥ 2) and low (Z < 2) APOC2 mRNA level. (D) Overall survival of ovarian serous cystadenocarcinoma patients with APOC2 mutations and high (Z ≥ 2) mRNA level versus wild type APOC2 and low (Z < 2) mRNA level. The differences between groups were analyzed by Mantel-Cox test.

AUTHOR CONTRIBUTIONS
Yuqiao Liu performed the analyses and generated the figures. Yiting Meng performed and wrote the expression analysis. Tian Zhang performed the survival analysis. Yuqiao Liu and Houda Alachkar wrote the manuscript. Houda Alachkar conceived the project, designed the research, and supervised the project.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study were derived from the following resources available in the public domain:
cBioPortal (https://www.cbioportal.org/)
Oncomine database (https://www.oncomine.org/resource/main.html)
NCBI-GEO database (https://www.ncbi.nlm.nih.gov/gds)
UALCAN database (http://ualcan.path.uab.edu/analysis.html)

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SUPPORTING INFORMATION
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