Crohn's disease-associated ATG16L1 T300A genotype is associated with improved survival in gastric cancer

Changqing Ma  
*University of Pittsburgh*

Chad E Storer  
*Washington University School of Medicine in St. Louis*

Uma Chandran  
*University of Pittsburgh*

William A LaFramboise  
*University of Pittsburgh*

Patricia Petrosko  
*University of Pittsburgh*

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Please let us know how this document benefits you.

**Recommended Citation**

Ma, Changqing; Storer, Chad E; Chandran, Uma; LaFramboise, William A; Petrosko, Patricia; Frank, Madison; Hartman, Douglas J; Pantanowitz, Liron; Haritunians, Talin; Head, Richard D; and Liu, Ta-Chiang, "Crohn's disease-associated ATG16L1 T300A genotype is associated with improved survival in gastric cancer." EBioMedicine. 67, 103347 (2021).  
https://digitalcommons.wustl.edu/open_access_pubs/10802

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Changqing Ma, Chad E Storer, Uma Chandran, William A LaFramboise, Patricia Petrosko, Madison Frank, Douglas J Hartman, Liron Pantanowitz, Talin Haritunians, Richard D Head, and Ta-Chiang Liu
Research paper

Crohn’s disease-associated ATG16L1 T300A genotype is associated with improved survival in gastric cancer

Changqing Ma\textsuperscript{a,*, b}, Chad E. Storer\textsuperscript{b}, Uma Chandran\textsuperscript{c}, William A. LaFramboise\textsuperscript{d,1}, Patricia Petrosko\textsuperscript{d,1}, Madison Frank\textsuperscript{a}, Douglas J. Hartman\textsuperscript{a}, Liron Pantanowitz\textsuperscript{a,2}, Talin Haritunians\textsuperscript{e}, Richard D. Head\textsuperscript{b}, Ta-Chiang Liu\textsuperscript{f,*}

\textsuperscript{a} Department of Pathology, University of Pittsburgh School of Medicine, 200 Lothrop Street, A-610, Pittsburgh, PA 15213, United States
\textsuperscript{b} Department of Genetics, Washington University School of Medicine, Saint Louis, MO 63110, United States
\textsuperscript{c} Department of Biomedical Informatics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, United States
\textsuperscript{d} UPMC Hillman Cancer Center, Cancer Genomics Facility, Pittsburgh, PA 15232, United States
\textsuperscript{e} F. Widjaja Family Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, United States
\textsuperscript{f} Departments of Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8118, Saint Louis, MO 63110, United States

\textsuperscript{a} Corresponding authors.
E-mail addresses: mac2@upmc.edu (C. Ma), tliu27@wustl.edu (T.-C. Liu).
\textsuperscript{1} Present address: Pathology and Laboratory Medicine, Allegheny Health Network, 320 East North Avenue, Pittsburgh, PA 15212.
\textsuperscript{2} Present address: Department of Pathology & Clinical Labs, University of Michigan, Ann Arbor, MI 48109.

\begin{abstract}
\textbf{Background:} A non-synonymous single nucleotide polymorphism of the ATG16L1 gene, T300A, is a major Crohn’s disease (CD) susceptibility allele, and is known to be associated with increased apoptosis induction in the small intestinal crypt base in CD subjects and mouse models. We hypothesized that ATG16L1 T300A genotype also correlates with increased tumor apoptosis and therefore could lead to superior clinical outcome in cancer subjects.

\textbf{Methods:} T300A genotyping by Taqman assay was performed for gastric carcinoma subjects who underwent resection from two academic medical centers. Transcriptomic analysis was performed by RNA-seq on formalin-fixed paraffin-embedded cancerous tissue. Tumor apoptosis and autophagy were determined by cleaved caspase-3 and p62 immunohistochemistry, respectively. The subjects’ genotypes were correlated with demographics, various histopathologic features, transcriptome, and clinical outcome.

\textbf{Findings:} Of the 220 genotyped subjects, 163 (74%) subjects carried the T300A allele(s), including 55 (25%) homozygous and 108 (49%) heterozygous subjects. The T300A/T300A subjects had superior overall survival than the other groups. Their tumors were associated with increased CD-like lymphoid aggregates and increased tumor apoptosis without concurrent increase in tumor mitosis or defective autophagy. Transcriptomic analysis showed upregulation of WNT/β-catenin signaling and downregulation of PPAR, EGFR, and inflammatory chemokine pathways in tumors of T300A/T300A subjects.

\textbf{Interpretation:} Gastric carcinoma of subjects with the T300A/T300A genotype is associated with repressed EGFR and PPAR pathways, increased tumor apoptosis, and improved overall survival. Genotyping gastric cancer subjects may provide additional insight for clinical stratification.

© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
\end{abstract}

1. Introduction

Our knowledge of cancer genetics has centered on oncogenes and tumor suppressor genes [1,2]. Recent studies have demonstrated that single nucleotide polymorphisms (SNPs) are also associated with susceptibility to sporadic cancers [2–5]. To date, genome-wide association studies (GWAS) have identified nearly a thousand predisposition variants significantly associated with over thirty malignancies [5–12]. These alterations have provided insights into new pathways for tumorigenesis and new targets for therapeutics [5]. Although most variants demonstrate a modest increase in risk [5], the combined effects of multiple SNPs are potentially useful in population-based cancer risk stratification and prevention programs [13,14]. Likewise, GWAS studies have also identified SNPs associated with response to chemotherapy [15], treatment-related toxicity [16], and clinical outcomes in cancer patients [17–19]. Therefore, identifying SNPs and other genetic alterations that correlate with clinical

https://doi.org/10.1016/j.ebiom.2021.103347
2352-3964/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)
Research in context

Evidence before this study

Single nucleotide polymorphisms can be associated with cancer susceptibility and/or prognosis. We previously showed that the ATG16L1 T300A variant, a major Crohn’s disease susceptibility allele, induces apoptosis (through repression of the PPAR-γ pathway) in the small intestinal crypt base in CD subjects. Notably, CD subjects with the T300A/T300A genotype have more aggressive disease course after surgical resection, whereas T300A/T300A subjects with colorectal carcinoma demonstrate superior prognosis.

Added value of this study

Our results demonstrate that the T300A/T300A genotype is associated with superior overall survival, increased tumor apoptosis and repressed EGFR and PPAR pathways in gastric cancer.

Implications of all the available evidence

Genotyping of genes and variants that are not involved in oncogenic or tumor suppressor pathways may provide insight in stratifying gastric cancer subjects.

outcome may provide novel insight into the pathobiology and management of cancer.

One area of which GWAS has provided insight into disease pathogenesis which resulted in mechanistic discoveries is immune-associated disorders [20]. Common SNPs/variants that increase the susceptibility for inflammatory bowel disease (IBD), asthma, rheumatoid arthritis, and systemic lupus erythematosus have been identified [11,20–25], and some have also been associated with increased cancer risk [26]. Lessons from these informative studies have fostered functional validations of selected SNPs. Most importantly, since these SNPs may exert effects in a wide spectrum of tissue and cell types [27], exploration of the role of these SNPs in other diseases such as malignancy may yield new hypotheses and/or insights into tumorigenesis through gene-specific, tissue-specific, or environment-specific mechanisms that merit testing.

Notably, some of the SNPs identified in immune-related disorders are also common in non-disease control subjects, suggesting that evolutionarily these SNPs may have acquired important roles in maintaining tissue homeostasis [27–29]. One such example is the ATG16L1 Thr300Ala (T300A) SNP, the most significant susceptibility SNP in the development of Crohn’s disease (CD) [30]. While originally described as a key execution member of the autophagy pathway [31], ATG16L1 is also known to affect multiple cell death pathways, including apoptosis [32] and necroptosis [33]. We and others have shown that Atg16l1 T300A mice showed defects in secretory cell lineages in the gut, resulting in altered immunity that affects pathogen clearance [29,34,35]. Notably, CD subjects harboring ATG16L1 T300A have more aggressive disease course after resection [32,36,37]. Interestingly, ATG16L1 T300A also confers protection from Escherichia coli infection [28], but promotes Helicobacter pylori infection [38]. Thus, it is likely that the effect of ATG16L1 T300A is organ-specific and disease-specific.

We previously showed that CD subjects and mice with ATG16L1 T300A possessed increased apoptosis (through repression of the PPAR-γ pathway) in the small intestinal crypt base [32]. Given the role of apoptosis as a major cell death pathway employed to target cancers [39], we hypothesized that ATG16L1 T300A may also confer increased tumor apoptosis, which could lead to improved survival in cancer. Herein we show that in gastric cancer, the T300A genotype is associated with unique histologic features, increased cancer apoptosis without concomitant increase in cancer proliferation or autophagy deficiency, and importantly, superior overall survival.

2. Methods

2.1. Gastric cancer cases

Gastric cancer cases resected between 2008 and 2012 were retrospectively collected by searching the surgical pathology archives of the University of Pittsburgh Medical Center (UPMC). Also included were gastric cancer cases resected between 1999 and 2013 from the Barnes-Jewish Hospital/Washington University. These cases were first described by Olsen et al. [40]. The University of Pittsburgh Medical Center and the Barnes-Jewish Hospital/Washington University are both large academic medical centers well-known in the United States. Cases from both institutions were collected in the aforementioned time frame to ensure the post-surgical follow-up period is long enough (at least 5 years) for survival analysis. For each subject / case from the UPMC, the electronic medical records were reviewed and the following demographic, clinical, and pathologic information were retrieved: gender, race, age at operation, pathologic and clinical stage, lymphovascular invasion, perineural invasion, status of surgical resection margins, pre- and post-surgery treatments if received, history of Helicobacter pylori infection, and survival. For cases from the Barnes-Jewish Hospital, the aforementioned demographic, clinical, and pathologic information were updated and only cases with up-to-date clinical follow-up information at the time of data collection were included in this study.

2.2. Ethics statement

This study was approved by the Institutional Review Boards of the University of Pittsburgh (STUDY20050115) and the Washington University (201301164). Informed consent from all participants was waived by the IRB committees.

2.3. Histologic evaluation

Tissue sections of the carcinoma for each case were reviewed without knowledge of the T300A genotype of each subject. Histologic subtyping for each tumor was performed using criteria of the Lauren classification system [41]. Specifically, a tumor is classified as intestinal-type if the carcinoma demonstrates intestinal differentiation with glandular / tubular structure formation. A tumor is classified as diffuse-type if the carcinoma cells demonstrate lack of cohesiveness (in other words lack of adhesion with each other) and infiltrating the gastric wall as single cells or small clusters of tumor cells. A tumor is classified as mixed-type if histologic features of both intestinal-type and diffuse-type carcinomas are observed in the same tumor.

Histologic grading of each case was performed according to criteria in the eighth edition of the American Joint Committee on Cancer recommendation on cancer staging [42]. This histologic grading system is based on the extent of glandular differentiation. In particular, a well-differentiated adenocarcinoma (G1) is defined as a tumor with greater than 95% of the tumor composed of glands. A moderately-differentiated adenocarcinoma (G2) is a tumor with 50% to 95% of the tumor composed of glands. A poorly-differentiated adenocarcinoma (G3) is a tumor with 49% or less of the tumor composed of glands. In this study, a tumor with mixed differentiation such as a mixed adenoneuroendocrine carcinoma was classified as histologic grade X (Gx).

The presence of a CD-like lymphoid reaction was evaluated as follows. For each case, all tumor sections were evaluated at low magnification to find one section with the most intra- and peri-tumoral lymphoid aggregates. In this tumor section, the number of intra- and peri-tumoral lymphoid aggregates were counted in 3 consecutive microscopic fields using 10× objective lens and 10× eye piece (the...
use of both in combination yields a 100× field of examination, which corresponds to a surface area of 3.8 mm² using an Olympus BX46 microscope). When on average the number of lymphoid aggregates was one or more (mean number of lymphoid aggregates per 100 × field ≥ 1), the tumor was scored as positive for CD-like lymphoid reaction.

Mitotic activity of each tumor was evaluated by counting the number of mitotic figures in the most mitotically active area in the tumor. Briefly, for each case, all tumor sections were first evaluated at low magnification to find a “hotspot” area with the most mitotic activity. In the “hotspot” area, the number of mitotic figures was then counted in 3 consecutive high-power fields (one high-power field = 400 × [40 × objective lens and 10× eye piece], which correspond to a surface area of 0–24 mm² using an Olympus BX46 microscope). The mean number of mitotic figures in one high-power field (400 ×) was calculated for each tumor.

2.4. Genotyping

Genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue using the QiAamp DNA FFPE tissue kit (Qiagen Inc., Valencia, CA, catalog number: 56404) and subsequently used for T300A genotyping with TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific, catalog number: 4351379) following the manufacturer’s instructions.

2.5. Immunohistochemistry and in-situ hybridization

For cleaved caspase-3 immunohistochemistry, unstained slides were deparaffinized, followed by antigen retrieval using Trilogy (Cell Marque, catalog number: 9209P-09) and subsequently used for T300A genotyping with TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific, catalog number: 4351379) following the manufacturer’s instructions.

2.6. Quantitative digital image analysis of CD8-positive or CD4-positive T cell density and CD56-positive NK cell density

The CD4, CD8, and CD56 stained slides were digitized using an Aperio AT2 scanner (Leica Biosystems, Buffalo Grove, IL), at 40× magnification. The CD4 immunostained slide and CD8 stained slide for each case were manually annotated to outline the areas of invasive adenocarcinoma. Non-neoplastic gastric tissue and areas of necrosis were specifically excluded from the area of analysis. The Aperio nuclear v9 algorithm, a component of the commercially available Leica/Aperio image analysis platform, was used to count the CD4- or CD8-stained cells as previously described [43]. The CD4-positive or CD8-positive T cell density was determined by dividing the number of CD4-positive or CD8-positive cells by the area (measured in mm²) examined. Densities (number of cells / mm²) of CD56-positive, CD4-positive, or CD8-positive cells in peri- and intratumoral lymphoid aggregates were additionally determined using the same algorithm.

2.7. Transcriptomic analysis

For each tumor included in transcriptomic analysis, a representative FFPE tumor tissue block was selected. The tumor area desired for transcriptomic analysis was manually circled on the hematoxylin and eosin stained tissue section. Manual microdissection of the desired tumor area was then performed on 6 to 10 unstained tumor tissue sections at 10 μm thickness for each tumor to ensure that only area of viable tumor but not necrosis or uninvolved tissue was included in transcriptomic analysis (Supplementary Fig. 1). Total RNA was extracted from manually dissected tumor tissue using the Qiagen miRNAeasy mini kit (Qiagen Inc., catalog number: 74104) according to the manufacturer’s recommendations. RNA library was prepared using the TrueSeq RNA ACCESS library preparation kit (Illumina, San Diego, CA, catalog number: 15049525). Transcriptomic analysis was performed on the NextSeq 500 system according to the manufacturer’s recommendations (please see Supplementary Method for details).

Quality of the RNAseq results of all 47 cases were assessed using FastQC (v0.11.5). All 47 cases fulfilled the quality control parameters. Samples were then aligned to the GRCh36 genome with HISAT2 (v2.1.0) [44] and to the human transcriptome with Salmon (v0.11.0) [45]. Alignments were quality assessed with QoRTs (v1.1.8) [46] and RNASEQC (v1.1.8) [47]. Gene expression was quantified with HTSeq (v0.9.0) [48]. Data normalization with the weighted trimmed mean of M-values (TMM) method and differential gene expression between T300A genotypes were performed with the edger R package [49]. Benjamini–Hochberg method was used for correction of multiple testing for differential gene expression.

Differentially expressed genes with P < 0.01 were further analyzed by a novel knowledge engine called ‘Comprehensive Multiomics Platform for Biological Interpretation’ (CompBio: https://www.percyai.com/CompBio) [50]. CompBio performs a literature analysis to identify relevant biological processes and pathways represented by the differentially expressed entities (genes, proteins, mRNA’s, or metabolites). This is accomplished with an automated Biological Knowledge Generation Engine that extracts all abstracts from PubMed that reference entities of interest (or their synonyms), using contextual language processing and a biological language dictionary that is not restricted to fixed pathway and ontology knowledge bases. Conditional probability analysis calculates the statistical enrichment of biological concepts (processes/pathways) over those that occur by random sampling. Related concepts built from the list of differentially expressed entities are further clustered into higher-level themes (e.g., biological pathways/processes, cell types and structures, etc.). The dataset was then cross-referenced with the T300A/T300A-specific transcriptomic themes identified in mouse ileum (ArrayExpress database E-MTAB-5707) [32].

2.8. Statistics

Statistical analysis was performed with GraphPad Prism 7 for Windows Version 7.00 (GraphPad Software, La Jolla, CA) and IBM SPSS (Release 23.0.0.0). GraphPad Prism 7 was used to perform both paired and unpaired Student t-tests, Mann-Whitney test, and Kruskal-Wallis test, which were used for comparison of continuous variables. IBM SPSS was used to perform survival analysis, Two-sided
Fisher’s exact test, and Chi-Square test. The latter two tests were used for the comparison of categorical data. Hardy–Weinberg equilibrium testing was performed by comparing observed genotype frequencies with those expected in Caucasian subjects and in African American subjects by Chi-square test in R using the package named Hardy–Weinberg (https://cran.r-project.org/web/packages/HardyWeinberg/HardyWeinberg.pdf). All tests were two-tailed and a P value less than 0.05 (P < 0.05) was considered significant. For survival analysis, overall survival was defined as the duration between surgical resection and either death or the latest clinical follow-up time. Death occurring within one month of the initial operation was attributed to operative mortality and was not included in the survival analysis. Subjects who were clinical stage IV at the time of surgery, i.e., with distant metastasis at the time of surgery, were considered never disease free and hence excluded from the analysis for disease-free survival. Survival analysis was performed using Cox proportional hazard model and Kaplan–Meier analysis with log-rank statistics. Potential confounding factors in univariate and/or multivariate analysis included race, histologic grade, histologic subtype by Lauren classification, clinical stage, surgical resection margin, lymphovascular invasion, perineural invasion, Helicobacter pylori infection, and chemotherapy.

Sequencing files are deposited at Gene Expression Omnibus (GEO) under accession number GSE152415.

2.9. Role of funding source

The funder (NIH) did not have any role in study design, data collection, data analyses, interpretation, or writing of report.

3. Results

3.1. Characteristics of the gastric cancer cohort

We retrospectively collected tissue from 220 consecutively resected gastric cancer subjects from two academic medical centers for genotyping and histopathology analysis. Among them, 163 (74%) subjects carried the T300A allele(s), including 55 (25%) homozygous T300A/wild-type (WT) subjects. Homozygous WT/WT genotype was detected in 57 (26%) subjects. The flow diagram of the gastric cancer cases analyzed in this study is shown in Fig. 1, and the demographics and clinicopathologic characteristics of the 220 genotyped subjects are listed in Table 1.

The T300A allele frequency was 0.56 and the WT allele frequency was 0.44 in Caucasian subjects in our cohort. In African American subjects the allele frequency was 0.56 and the WT allele frequency was 0.44 in Caucasian subjects and African American subjects did not deviate from Hardy–Weinberg equilibrium (P > 0.05 [Chi-Square test]). Since the T300A allele is more frequent in Caucasians than in African American subjects, homozygous T300A/T300A genotype was more frequently identified in Caucasians than other genotypes (P < 0.01 [Fisher’s exact test]; Table 1). Only 33 (15%) subjects had either clinical history or active/concurrent Helicobacter pylori infection. When stratified by genotype, Helicobacter pylori infection was more often seen in homozygous WT/WT subjects than in subjects with other genotypes (14/57, 25% versus 11/108, 10% versus 8/55, 15%). However, this difference was not statistically significant (P = 0.07 [Fisher’s exact test]; Table 1). There was also no significant difference in the histologic degree of tumor differentiation, histologic type by the Lauren classification [41], clinical stage, receipt of pre- and post-surgical treatment, or other histopathologic parameters between different genotype groups (P > 0.05 for all [Fisher’s exact test]; Table 1).

3.2. Gastric cancer subjects with ATG16L1 T300A/T300A showed superior prognosis after surgery

We next assessed if the ATG16L1 T300A genotype correlated with clinical outcome. Among all 220 subjects with confirmed genotypes, those with the T300A/T300A genotype showed superior overall survival compared to the other two groups combined (WT/T300A and WT/WT) by Kaplan–Meier survival analysis with log-rank statistics (mean, standard error, and 95% confidence interval [CI]) of overall survival: 107 months, 14 months, [80–135] versus 64 months, 9 months, [52–76], P = 0.035 (Fig. 2a). Using Cox proportional hazards modeling, however, the T300A/T300A genotype was not an independent predictor of overall survival for the entire cohort in multivariate analysis (multivariate analysis hazard ratio and 95% CI: 0.77, 0.47 – 1.26, P = 0.31) (Supplementary Table 1).

In order to exclude potential confounding effects on overall survival introduced by pre- and post-surgical chemotherapy, we next performed survival analysis in the subset of 126 subjects who received only surgical treatment. Among them, subjects with T300A/T300A genotype had significantly longer overall survival than subjects with WT/T300A and WT/WT genotypes combined using Kaplan–Meier survival analysis with log-rank statistics (mean, standard error, and 95% CI: 164 months, 16 months, [132–196] versus 60 months, 6 months, [51–69], P = 0.019) (Fig. 2b). Using Cox proportional hazards modeling, the T300A/T300A genotype was associated with a significant increase in overall survival by both univariate and multivariate analysis (multivariate analysis hazard ratio and 95% CI: 0.31, 0.11 – 0.88, P = 0.03) (Table 2).

Approximately one-third (45, 36%) of the 126 subjects with surgery as the only treatment died during the follow-up period. WT/WT subjects had significantly higher mortality than subjects with other T300A genotypes (WT/WT: 19/35, 54% versus WT/T300A: 20/57, 35% versus T300A/T300A: 6/30, 20%, P = 0.02 [Fisher’s exact test]). The percentages of patients who died of disease progression were similar between the three groups: 7/19 (37%) in WT/WT, 7/20 (35%) in WT/T300A, and 2/6 (33%) in T300A/T300A groups (P = 0.73 [Fisher’s exact test]). Likewise, other comorbidities and surgical complications accounted for death in 12/19 (63%) WT/WT, 13/20 (65%) in WT/T300A, and 4/6 (67%) in T300A/T300A groups (Supplementary Table 2).

3.3. T300A/T300A genotype was associated with unique histological features

We and others have previously found that T300A/T300A genotype was associated with histomorphologic changes in CD subjects and mouse models [34,51,52], including increased apoptosis induction in
small intestinal crypt base where Paneth cells and intestinal stem cells [both are CD-relevant cell types] reside [53]. We therefore hypothesized that T300A/T300A genotype was also associated with unique histologic features in gastric cancer. To exclude the possibility that neoadjuvant therapy may affect histologic features, we performed histologic assessments of the tumors from subjects who did not receive neoadjuvant therapies, including subjects who received no additional treatment either before or after surgery and those who received only adjuvant treatment. Cases with tumor blocks available (n = 119) were included in histologic evaluation (Fig. 1). As shown in Supplementary Table 3, there was no significant difference in Helicobacter pylori infection, the histologic degree of tumor differentiation, histologic subtype, clinical stage, or other histologic features between different genotype groups (P > 0.05 for all [Fisher’s exact test]).

In contrast, we found that the T300A/T300A tumors were more likely to contain intra- and peri-tumoral CD-like lymphoid aggregates than homozygous WT/WT tumors [25/28, 89% versus 21/32, 66%, P = 0.037 [Fisher’s exact test]] (Fig. 3a, b, and Table 3). We further analyzed the density of lymphocytes not associated with lymphoid aggregates, using an unbiased automated image analysis system [43]. There was no difference in the density of CD8-positive or CD4-positive T cells at the tumor invasive front (tumor-stroma interface) (P = 0.60 and 0.89, respectively, [Kruskal–Wallis test]) or within the tumors (P = 0.72 and 0.61, respectively, [Kruskal–Wallis test]) (Supplementary Table 4 and Supplementary Fig. 2). We additionally analyzed the density of CD4-positive T cells, CD8-positive T cells, and CD56-positive NK cells in peri- and intratumoral lymphoid aggregates using the same automatic image analysis system [43]. These lymphoid aggregates contained similar proportions of CD4-positive T cells and CD8-positive T cells (mean and standard deviation: CD4-positive T cell density: 1587 ± 76; CD8-positive T cell density: 1556 ± 61; P = 0.78 [paired t-test]). The ratio of CD4-positive cell density and CD8-positive cell density was not associated with tumor T300A genotypes (mean and standard deviation of CD4 cell density / CD8 cell density: WT/WT: 1.06 ± 0.38 versus T300A/T300A: 1.11 ± 0.76; P = 0.63 [Mann–Whitney test]) (Supplementary Figs. 3a, c, and d). In contrast CD56-positive NK cell was not a significant component in peri- and intratumoral lymphoid aggregates (Supplementary Figs. 3b and e).

### 3.4. T300A/T300A genotype was associated with increased tumor apoptosis

Given the role of ATG16L1 T300A in mediating various cell death pathways, predominantly autophagy and apoptosis, we next determined if proliferation, autophagy, and apoptosis were differentially correlated with subject genotypes. We found that there was no difference in the mitotic activities in the tumors of the T300A/T300A group compared to the others (P = 0.64 [Kruskal–Wallis test]) (Supplementary Figs. 4a, b, and Supplementary Table 4). Likewise, as a

| Table 1 | Demographics and clinicopathologic characteristics stratified by T300A genotype. |
|---------|---------------------------------------------------------------------------------|
|          | All cases (N = 220) | WT/WT (N = 57) | WT/T300A (N = 108) | T300A/T300A (N = 55) | P     |
| Age (year; median, [IQR]) | 73 (19) | 75 (15) | 71 (20) | 72 (22) | 0.40 |
| Gender (n, %) | Male | 124 (56) | 29 (51) | 60 (56) | 35 (64) | 0.38 |
| | Female | 96 (44) | 28 (49) | 48 (44) | 20 (36) | 0.38 |
| Race (n, %) | Caucasian | 135 (81) | 24 (59) | 71 (87) | 40 (71) | < 0.01 |
| | African American | 20 (12) | 13 (32) | 5 (6) | 2 (5) | 0.10 |
| | Others | 12 (7) | 4 (10) | 6 (7) | 2 (5) | 0.10 |
| Histologic type (n, %) | Intestinal | 104 (47) | 31 (54) | 45 (42) | 28 (51) | 0.48 |
| | Diffuse | 84 (40) | 18 (32) | 49 (45) | 20 (36) | 0.48 |
| | Mixed and other types | 29 (13) | 8 (14) | 14 (13) | 7 (13) | 0.48 |
| Histologic grade (n, %) | G1 | 14 (6) | 2 (4) | 7 (6) | 5 (9) | 0.14 |
| | G2 | 57 (26) | 21 (37) | 20 (19) | 16 (29) | 0.14 |
| | G3 | 146 (66) | 33 (58) | 79 (73) | 34 (62) | 0.14 |
| | Gx | 3 (1) | 1 (2) | 1 (1) | 0 | 0 |
| Clinical stage (n, %) | I | 72 (33) | 17 (30) | 31 (29) | 24 (44) | 0.10 |
| | II | 58 (26) | 20 (35) | 25 (23) | 13 (24) | 0.10 |
| | III | 62 (28) | 16 (28) | 32 (30) | 14 (25) | 0.10 |
| | IV | 26 (12) | 3 (5) | 19 (18) | 4 (7) | 0.10 |
| | Unknown | 2 (1) | 1 (2) | 1 (1) | 0 | 0 |
| Positive resection margin (n, %) | 34 (15) | 8 (14) | 20 (19) | 6 (11) | 0.44 |
| Positive lymphovascular invasion (n, %) | 135 (61) | 33 (58) | 71 (66) | 31 (56) | 0.31 |
| Positive perineural invasion (n, %) | 93 (42) | 21 (37) | 52 (48) | 20 (36) | 0.29 |
| Helicobacter pylori infection (n, %) | 33 (15) | 14 (25) | 11 (10) | 8 (15) | 0.07 |
| Vital status (at the time of data collection) (n, %) | Alive | 93 (43) | 21 (37) | 43 (40) | 29 (53) | 0.24 |
| | Death | 121 (55) | 34 (60) | 61 (56) | 26 (47) | 0.24 |
| | Lost to follow-up | 6 (3) | 2 (4) | 4 (4) | 0 | 0 |
| Overall follow-up time (month; median, [IQR]) | 61 (10) | 8 (14) | 20 (19) | 6 (11) | 0.44 |
| Recurrence (at the time of data collection) (n, %) | 135 (61) | 33 (58) | 71 (66) | 31 (56) | 0.31 |
| | Yes | 62 (28) | 16 (28) | 32 (30) | 14 (25) | 0.31 |
| | No | 124 (56) | 29 (51) | 60 (56) | 35 (64) | 0.31 |
| Disease free time (month; median, [IQR]) | 23 (46) | 18 (45) | 22 (48) | 25 (44) | 0.37 |
| Chemotherapy (n, %) | None | 126 (57) | 36 (63) | 60 (56) | 30 (55) | 0.79 |
| | Neoadjuvant only | 13 (6) | 2 (4) | 7 (6) | 4 (7) | 0.79 |
| | Adjuvant only | 46 (21) | 13 (23) | 23 (21) | 10 (18) | 0.79 |
| | Both | 35 (16) | 6 (11) | 18 (17) | 11 (20) | 0.79 |

Abbreviations: WT: wild-type; IQR: interquartile range; G: grade. Fisher’s Exact Test was used for the comparison of categorical data.

Kruskal–Wallis test was used for comparison of continuous variables. Fisher’s Exact Test was used for the comparison of categorical data.
readout for autophagy activation, the prevalence of p62-positive immunohistochemical staining was similar between all groups (P = 0.27 [Chi-Square test]) (Supplementary Figs 4c, d, and Supplementary Table 4). In contrast, the T300A/T300A tumors showed significantly increased intratumoral apoptosis compared with tumors of other genotypes (P = 0.04 [Kruskal–Wallis test]) by cleaved-caspase 3 immunohistochemistry (Figs. 3c, d, e, f, and Table 3). Therefore, the T300A/T300A genotype was associated with increased intratumoral apoptosis without concurrent changes in autophagy activation or mitosis.

3.5. Gastric cancer from T300A/T300A subjects showed unique transcriptomic signatures

To further characterize the pathways that may be involved in mediating the apoptosis induction in the T300A/T300A tumors, we performed global transcriptomics on a subset of 47 tumors that did not receive neoadjuvant therapies (including 13 WT/WT, 17 WT/T300A, and 17 T300A/T300A cases) (Fig. 1). Of note, since gastric carcinoma with DNA mismatch repair (MMR) protein deficiency

Table 2

Univariate and multivariate analysis of overall survival in subjects without additional treatment (N = 126) by Cox regression.

| No additional treatment cases (N = 126) | Univariate analysis | Multivariate analysis |
|----------------------------------------|---------------------|----------------------|
|                                        | P       | HR  | 95% CI    | P       | HR  | 95% CI    |
| T300A/T300A                            | 0.02    | 0.37 | 0.16–0.88 | 0.03    | 0.31 | 0.11–0.88 |
| African American                       | 0.31    | 0.61 | 0.24–1.58 | 0.23    | 1.58 | 0.74–3.40 |
| High histologic grade                  | 0.71    | 0.89 | 0.49–1.64 | 0.88    | 0.88 | 0.49–1.64 |
| Diffuse type histology                 | 0.59    | 0.82 | 0.40–1.68 | 0.59    | 0.82 | 0.40–1.68 |
| Stage III & IV                         | < 0.001 | 6.0  | 2.98–12.24 | 0.02    | 3.0  | 1.16–7.74 |
| Positive surgical resection margin     | 0.01    | 2.85 | 1.24–6.56 | 0.96    | 0.97 | 0.32–2.94 |
| Lymphovascular invasion                | < 0.001 | 5.45 | 2.57–11.57 | < 0.001 | 4.11 | 1.86–9.07 |
| Perineural invasion                    | 0.04    | 1.96 | 1.04–3.72 | 0.71    | 1.17 | 0.52–2.61 |
| Helicobacter pylori infection          | 0.51    | 0.74 | 0.31–1.78 | 0.61    | 0.74 | 0.31–1.78 |

Abbreviations: HR: hazard ratio; CI: confidence interval.
Fig. 3. Tumors from T300A/T300A subjects showed more lymphoid aggregates and apoptosis. (a) T300A/T300A tumors were more likely to have intra- and peritumoral lymphoid aggregates than WT/WT tumors \((P = 0.037 \text{ Fisher’s exact test})\) (WT/WT \(n = 32\), WT/T300A \(n = 55\), T300A/T300A \(n = 28\)). (b) Representative photomicrograph of a gastric adenocarcinoma with intra- and peritumoral lymphoid aggregates (arrows). (c) Representative photomicrograph of a WT/WT tumor with 6% of cleaved-caspase 3-positive (CC3+) tumor cells (arrows) / 400× field on average in 5 representative fields. (d) Representative photomicrograph of a WT/T300A tumor with 6% of CC3+ tumor cells (arrows) / 400× field on average in 5 representative fields. (e) Representative photomicrograph of a T300A/T300A tumor with 10% of CC3+ tumor cells (arrows) / 400× field on average in 5 representative fields. (f) T300A/T300A tumors had significantly increased CC3+ tumor cells compared with tumors of the other two genotypes \((P = 0.04 \text{ by ANOVA})\) (WT/WT \(n = 30\), WT/T300A \(n = 30\), T300A/T300A \(n = 26\)). Error bars represent mean with 95% confidence interval. Scale bars: b-200 μm; c, d, e-50 μm.

Table 3
Tumor immune microenvironment and apoptosis by cleaved-caspase3 immunostaining stratified by T300A genotype.

|                        | WT/WT | WT/T300A | T300A/T300A | \(P^1\) | \(P^2\) | \(P^3\) |
|------------------------|-------|----------|-------------|--------|--------|--------|
| Peri- and intra-tumoral lymphoid aggregates (n, %) | N = 32 | N = 55 | N = 28 |        |        |        |
| Negative (< 1 / 100 × field) | 11 (34) | 10 (18) | 3 (11) | 0.06 | 0.18 | 0.037 |
| Positive (≥ 1 / 100 × field) | 21 (66) | 45 (82) | 25 (89) |        |        |        |
| No. of cases stained for CC3 | N = 30 | N = 30 | N = 26 |        |        |        |
| % of CC3-positive tumor cells / 400× field (median, [IQR]) | 4.7 (3.1) | 5.2 (3.5) | 6.8 (5.5) | 0.04 | 0.01 | 0.04 |

Kruskal–Wallis test and Mann–Whitney test were used for comparison of continuous variables. Fisher’s exact test was used for the comparison of categorical data.

Abbreviations: WT: wild-type; IQR: interquartile range; CC3: cleaved-caspase3.

\(1\) \(P\)-value for the comparison between all three groups.

\(2\) \(P\)-value for the comparison between WT/WT and WT/T300A combined and T300A/T300A (WT/WT & WT/T300A vs. T300A/ T300A).

\(3\) \(P\)-value for the comparison between WT/WT and T300A/T300A.
Transcriptomic analysis identified gene signatures associated with T300A/T300A genotype in gastric cancer. (a) Intensity plot of normalized gene expression level for 487 significantly expressed genes (\(P < 0.001\)) associated with the T300A/T300A genotype (WT/WT & WT/T300A \(n = 30\), T300A/T300A \(n = 17\)). (b) Transcriptomic themes from genes upregulated in the T300A/T300A tumors performed by CompBio analysis. (c) Themes from genes downregulated in the T300A/T300A tumors performed by CompBio analysis.
small cell lung cancers [55,58]. Our study suggests that the protective effect of the T300A may be common to multiple cancer types, although the mechanisms by which T300A confers improved survival may be organ-specific. In particular, the T300A/T300A genotype was not associated with a decrease in clinical stage IV/metastatic gastric carcinoma in our cohort. More importantly, the main molecular pathways associated with T300A in gastric carcinoma may be different from pathways identified in colon cancer. Type I IFN activation was detected in human colon cancer cell line with T300A mutation [55] but was not detected in gastric carcinoma with T300A/T300A genotype.

Notably, the ATG16L1 T300A variant has been associated with susceptibility of gastric cancer. In the study by Burada et al., the risk of developing gastric carcinoma was significantly reduced (odds ratio: 0.52, 95% confidence interval 0.13–0.88, P = 0.013) in subjects carrying the T300A allele compared with those with WT/WT genotype in a cohort of 350 Romanian subjects [59]. In contrast, the T300A allele was associated with an increased risk of gastric carcinoma (adjusted odds ratio: 2.38, 95% confidence interval 1.34–4.24, P = 0.003) in 304 ethnic Chinese subjects [60]. These findings suggest that the ATG16L1 T300A allele may be associated with susceptibility of gastric cancer but its impact may be population specific. Additionally, studies performed in Crohn’s disease have shown that the genetics underlying disease prognosis may be independent of the genetics underlying disease susceptibility [61]. Further validation of the association between T300A genotype and gastric cancer susceptibility will ideally require studying large numbers of subjects, as has been performed in other disease types [3–5,30,61–64]. However, our findings together with results in colon and non-small cell lung cancers may imply a survival benefit of the T300A allele against carcinoma. In addition, we feel, this protective effect may be generalizable to wider populations given the relatively large number of subjects from different geographic areas included in our study (St. Louis and Pittsburgh, U.S.) and reports personal fees from Ibex.

T-C. L. was funded by NIH grants R01 DK125296 and R01 DK124274.

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103347.

Acknowledgments

The authors wish to thank members of the In Situ Hybridization and Developmental Laboratory of the Department of Pathology, University of Pittsburgh for excellent technical support. The authors wish to thank members of the Image Analysis Lab of the Department of Pathology, University of Pittsburgh, especially Ms. Lindsey Seigh, Mr. Matthew O’Leary, and Mr. Jon Duboy, for excellent technical support.

This study utilized the University of Pittsburgh Hillman Cancer Center shared resource facility (Cancer Genomics Facility) supported in part by award P30CA047904 (Dr. Laframboise and Dr. R Ferris). This study used the University of Pittsburgh Health Sciences Core Research Facilities (HSCRF) Genomics Research Core services.

T-C. L. was funded by NIH grants R01 DK125296 and R01 DK124274.

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2021.103347.

References

[1] Tomasetti C, Marchionni L, Nowak MA, Parmigiani G, Vogelstein B. Only three driver gene mutations are required for the development of lung and colorectal cancers. Proc Natl Acad Sci USA 2015;112:118–23.

[2] Bailey MH, Tokheim C, Porta-Pardo E, Sengupta S, Bertrand D, Weerasinghe A, et al. Comprehensive characterization of cancer driver genes and mutations. Cell 2018;173:371–85 e18.
[3] Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer–analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 1997;336:78–85.

[4] Fletcher O, Houlston RS. Architecture of inherited susceptibility to common cancer. Nat Rev Cancer 2010;10:353–61.

[5] Sud A, Kaminersky B, Houlston RS. Genome-wide association studies of cancer: disseminating insights and perspectives. Nat Rev Cancer 2010;10:792–704.

[6] Abecasis GR, International LDNA, Neale BD, Zhang H, Ziv E, Shao X, et al. A shared susceptibility locus in PCOS1 at 1q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. Nat Genet 2010;42:764–6.

[7] Helgasen H, Rafnsson TF, Olufsen HS, Jonsson JG, Sigurdsson A, Stacey SN, et al. Loss-of-function variants in ATM confer risk of gastric cancer. Nat Genet 2015;47:906–10.

[8] Shi Y, Hu Z, Wu C, Lin J, Li H, Dong J, et al. A genome-wide association study identifies a susceptibility locus for non-cardia gastric cancer at 3q13.31 and 3p13.1. Nat Genet 2011;43:1215–8.

[9] Mocelin S, Verdi D, Pooler KA, Nitti D. Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. Gut 2015;64:1209–19.

[10] Milne RL, Kuchenbaecker KB, Verhaak RG, Lussoni M, Easton DF, et al. Identification of ten variants associated with risk of estrogen receptor-negative breast cancer. Nat Genet 2017;49:1767–78.

[11] Noguchi E, Sakamoto H, Hirota T, Ochiai K, Imoto Y, Sakashita M, et al. Genome-wide association study identifies HLA-DP as a susceptibility gene for pediatric asthma in Asian populations. PLoS Genet 2011;7:e1002170.

[12] Huang KL, Masl NJ, Wu R, Dittert WJ, Wang J, Oh C, et al. Pathogenic germine variants in 10,389 adult cancers. Cell 2018;173:355–70.

[13] Lazenby JA, Silverman H, Barrierdeke VB, Barrowdale D, Dennis J, McGuffey L, et al. Prediction of breast and prostate cancer risks in male BRCA1 and BRCA2 mutation carriers using polygenic risk scores. J Clin Oncol 2017;35:2240–50.

[14] Shi Z, Yu T, Wu Y, Lin X, Bao Q, Jia H, et al. Systematic evaluation of cancer-specific genetic risk score in colorectal cancer in the cancer genome atlas and electronic medical records and genomics cohorts. Cancer Med 2019;8:3196–205.

[15] Johnson N, De Iese P, Migliorini G, Orr N, Broderick P, Catovsky D, et al. Cytomegalovirus-associated lymphoproliferative disease in patients with cancer: a multi-centre study. Lancet 1993;342:699–702.

[16] Shi Y, Hu Z, Wu C, Dai J, Feng J, et al. A genome-wide association study identifies a susceptibility locus for childhood cancer. Nat Genet 2011;43:1215–8.

[17] Mocelin S, Verdi D, Pooler KA, Nitti D. Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. Gut 2015;64:1209–19.

[18] Milne RL, Kuchenbaecker KB, Verhaak RG, Lussoni M, Easton DF, et al. Identification of ten variants associated with risk of estrogen receptor-negative breast cancer. Nat Genet 2017;49:1767–78.

[19] McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, et al. A three-stage genome-wide association study identifies a susceptibility locus for lung cancer. Nature 2013;502:417–9.

[20] Hartman DJ, Ahmad F, Ferris RL, Rimm DL, Pantanowitz L. Utility of CD8 score by automated quantitative image analysis in head and neck squamous cell carcinoma. Oral Oncol Res 2018;26:416–24.

[21] Kim D, Langned B, Salsberg S, Hitot, a fast spliced aligner with low memory requirements. Nat Methods 2015;12:357–60.

[22] Pato R, Duggal G, Love MI, Frizzay RA, Kingsford C. Salmon provides fast and bias-aware quantification of transcript expression. Nat Methods 2016;13:717–20.

[23] Hartley SW, Mulliken JC, QoRTs: a comprehensive toolset for quality control and data processing of RNA-Seq experiments. BMC Bioinform 2015;16:224.

[24] Collado V, Levin J, Parmar N, Hudders C, McPherson N, Krawczak M, et al. RNA-Seq: RNA-seq metrics for quality control and process optimization. Bioinformatics 2012;28:1530–2.

[25] Anders S, Pyl PT, Huber W. HTSeq—a python framework to work with high-throughput sequencing data. Bioinformatics 2015;31:169–70.

[26] Robinson MD, McCarthy DJ, Smyth GK. edgeR: a bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010;26:139–40.

[27] Agrawal CA, Ahern PP, Kung V, Hibberd MC, Cheng J, Gargule J, et al. Mechanisms by which sialylated milk oligosaccharides impact bone biology in a gnotobiotic mouse model of infant nutrition. Proc Natl Acad Sci USA 2011;108:25120–5.

[28] Clark J, K, Liu JY, Brown SL, Miyoshi H, Loh J, Jennewein J, et al. A key role for autophagy and the autophagy gene ATG16L1 in mouse and human intestinal Paneth cells. Nature 2008;456:259–63.

[29] Patel K, Miyoshi H, Beatty WL, Head RD, Malvin NP, Cadwell K, et al. Autophagy promotes control goblet cell function by potentiating reactive oxygen species production. EMBO J 2013;32:3130–44.

[30] Murphy A, Ly T, Peng I, Reichelt M, Katakam AK, Noubade R, et al. A three-stage genome-wide association study identifies new susceptibility loci for non-small cell lung cancer. Nat Genet 2015;47:1079–85.

[31] Bass AJ, Thorsson V, Shimulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014;513:262–3.

[32] Gordon WA, Messer JS, Murphy SF, Nero T, Lodelce DP, Weber CR, et al. The Thr300Ala variant in ATG16L1 is associated with improved survival in human colorectal cancer and enhanced production of type I interferon. Gut 2017;65:456–66.

[33] Cleynen I, Gonzalez JR, Figueroa C, Franke A, McGranahan N, Bortlik M, et al. Genetic factors conferring an increased susceptibility to develop Crohn’s disease also influence disease phenotype: results from the IBDChip European Project. Gut 2016;65:292–300.

[34] Varma M, Kadoji M, Leftkovitch A, Conway KL, Gao K, Mohanan V, et al. Cell type and stimulation-dependent transcriptional programs regulated by ATG16L1 and its Crohn’s disease risk variant T300A. J Immunol 2020;205:414–24.

[35] Qiao J, Liu H, Huang T, Yang Y, Liu B, Peng P, et al. The Thr300Ala variant in ATG16L1 is associated with decreased risk of brain metastasis in patients with non-small cell lung cancer. Autophagy 2017;13:1053–63.

[36] Burada F, Ciurea ME, Nicoli R, Streata I, Vilcea ID, Rogoveanu I, et al. ATG16L1 deficiency in the autophagy gene Atg16L1 enhances resistance to enteric pathogens. Cell 2017;169:55–67.

[37] Liu T-C, Kern JT, VanDussen KL, Xiong S, Kaiko GE, Wilen CB, et al. Interaction between smoking and ATG16L1T300A triggers Paneth cell defects in Crohn’s disease. J Clin Invest 2018;128:5110–22.
[61] Lee JC, Biasci D, Roberts R, Gearry RB, Mansfield JC, Ahmad T, et al. Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn’s disease. Nat Genet 2017;49:262–8.

[62] Brant SR, Okou DT, Simpson CL, Cutler DJ, Haritunians T, Bradfield JP, et al. Genome-wide association study identifies African-specific susceptibility loci in African Americans with inflammatory bowel disease. Gastroenterology 2017;152:206–17 e2.

[63] Cleynen I, Boucher G, Jostins L, Schumm LP, Zeissig S, Ahmad T, et al. Inherited determinants of Crohn’s disease and ulcerative colitis phenotypes: a genetic association study. Lancet 2016;387:156–67.

[64] Rivas MA, Avila BE, Koskela J, Huang H, Stevens C, Pirinen M, et al. Insights into the genetic epidemiology of Crohn’s and rare diseases in the Ashkenazi Jewish population. PLoS Genet 2018;14:e1007329.

[65] Camargo MC, Kim WH, Chiaravalli AM, Kim KM, Corvalan AH, Matsuo K, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis. Gut 2014;63:236–43.

[66] Pietrantonio F, Miceli R, Raimondi A, Kim YW, Kang WR, Langley RE, et al. Individual patient data meta-analysis of the value of microsatellite instability as a biomarker in gastric cancer. J Clin Oncol 2019;37:3392–400.

[67] Chang WH, Lai AG. The pan-cancer mutational landscape of the PPAR pathway reveals universal patterns of dysregulated metabolism and interactions with tumor immunity and hypoxia. Ann NY Acad Sci 2019;1448:65–82.

[68] Chen L, Peng J, Wang Y, Jiang H, Wang W, Dai J, et al. Fenofibrate-induced mitochondrial dysfunction and metabolic reprogramming reversal: the anti-tumor effects in gastric carcinoma cells mediated by the PPAR pathway. Am J Transl Res 2020;12:428–46.