An Autophagy-Related Gene Signature Predicts Prognosis of Early Stage Colorectal Cancer

Yi-feng Zou

Sun Yat-sen University Sixth Affiliated Hospital

Qiu-ning Wu

Sun Yat-sen University Sixth Affiliated Hospital

Si Qin

Sun Yat-sen University Sixth Affiliated Hospital

Xi Xi Chen

Sun Yat-sen University Sixth Affiliated Hospital

Jia-wei Cai

Sun Yat-sen University Sixth Affiliated Hospital

Ying-xin Tan

Sun Yat-sen University Sixth Affiliated Hospital

Jing-rong Weng

Sun Yat-sen University Sixth Affiliated Hospital

Yu-ming Rong

Sun Yat-sen University Cancer Center

Xutao Lin (✉ linxutao@mail2.sysu.edu.cn)

Sun Yat-sen University Sixth Affiliated Hospital <https://orcid.org/0000-0002-7110-3459>

Research article

Keywords: colorectal cancer, prognosis, autophagy-related gene, early stage, disease-free survival

Posted Date: August 26th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-59367/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background and aims

A certain number of early-stage colorectal cancer (CRC) patients suffer tumor recurrence after initial curative resection. In this context, an effective prognostic biomarker model is constantly in need. Autophagy exhibits a dual role in tumorigenesis. Our study aims to develop an autophagy-related gene (ATG) signature based on high-throughput data analysis for disease-free survival (DFS) prognosis of patients with stage I/II CRC.

Methods

Gene expression profiles and clinical information of CRC patients extracted from four public datasets were divided into training cohort (GSE39582, n=566), validation cohort (TCGA CRC, n=624), and meta-validation cohort (GSE37892 and GSE14333, n=420). Autophagy genes significantly associated with prognosis were identified.

Results

Among 655 autophagy-related genes, a 10 gene ATG signature, which was significantly associated with DFS in the training cohort (HR, 2.76[1.56–4.82]; $P=2.06\times 10^{-4}$), was constructed. The ATG signature, stratifying patients into high and low autophagy risk groups, was validated in the validation (HR, 2.29[1.15–4.55]; $P=1.5\times 10^{-2}$) and the meta-validation cohorts (HR, 2.5[1.03–6.06]; $P=3.63\times 10^{-2}$), and proved to be prognostic in a multivariate analysis. Functional analysis revealed enrichment of several immune/ inflammatory processes in the high autophagy risk group, where significantly higher infiltration of T regulatory cells (Tregs) was observed.

Conclusions

Our study established a prognostic ATG signature that effectively predicted DFS for early-stage CRC patients. Meanwhile, the study also revealed the possible correlation between autophagy and immune/ inflammatory processes and tumorigenesis.

1 Background

CRC is currently the second leading cause of cancer deaths worldwide, ranking third in morbidity[1]. Depending upon the tumor stage at diagnosis, relative 5-year survival rates for patients with CRC range from 65% for all stages to 91%, 82%, and 12% for patients diagnosed with stage I, II, and IV, respectively[2]. Although increasing awareness of cancer screening and advances in technology have improved early detection[3] and treatment without chemotherapy, around one-third of patients with stages I-III CRC still encounter tumor relapse after curative treatment[4]. Accordingly, there is a growing need for novel molecular markers for prognosis patterns, which might provide valuable information for supplementary adjuvant chemotherapy or other targeted therapy. Recently, for example, the predictive potency of KRAS and BRAF mutations, microsatellite instability (MSI) status, and CpG island methylator phenotype (CIMP) status in CRC had been studied extensively[3, 5].

Autophagy plays a dual role in tumorigenesis[6]. It inhibits early tumor initiation by the clearance of damaged mitochondria, peroxisome, and other cytotoxic substance, and also caters to the high metabolic demands from accelerated proliferating tumors by degrading intracellular organelle and macromolecule substances[7, 8]. To further investigate how autophagy affects the prognosis of early-stage CRC patients, we identified an ATG signature from CRC-specific transcriptomes based on high-throughput data analysis. The ATG signature, which stratified the stage I/II CRC patients into distinct risk groups, together with functional and pathway analysis, might provide insights into the mechanism of CRC recurrence and targeted treatment.

2 Methods

2.1 Public datasets

Gene expression data of CRC tissue samples and corresponding clinical information obtained from Gene Expression Omnibus (GEO <http://www.ncbi.nlm.nih.gov/geo/>) and Firehose Broad GDAC portal (<http://gdac.broadinstitute.org/>) were retrospectively analyzed. A total of 1610 patient samples from four independent public cohorts were included. The GSE39582 dataset (n=566) was intended for training, among which 309 patients without chemotherapy adjuvant but with survival information served as the discovery cohort. The Cancer Genome Atlas (TCGA) CRC dataset (n=624) was used for independent validation, while the GSE37892 and GSE14333 dataset (n=420) were combined for meta-validation. Transcripts per million (TPM) of level 3 RNA-Seq data in the log2 scale were applied to

calibrate the gene expression levels in the TCGA cohort. The 'combat' algorithm of the R package 'sva' and the z-scores were used to correct the batch effects, to standardize microarray data across multiple experiments, and compare them independent of the original hybridization intensities.

2.2 Construction of a prognostic autophagy-related gene (ATG) signature

To construct a prognostic ATG signature, we focused on 655 ATGs from 8 gene sets identified via MSigDB (version 6.2)[9-11] with the keyword "autophagy". Only 617 genes measured on all platforms involved in this study with high variation (determined by median absolute deviation (MAD) >0.5) were selected. After 1000 times of random Cox univariate regressions, genes with repeated significance, which indicated a strong relationship between ATGs and patients' DFS, were selected as candidates for the signature. A Cox proportional hazards regression model on CRC samples together with the least absolute shrinkage and selection operator (LASSO) were applied to minimize the risk of over-fitting as well as to generate a risk model.

Patients were divided into low and high autophagy risk groups in accordance with the optimal ATG signature cutoff, which was defined by the time-dependent receiver operating characteristic (ROC) curve analysis at 5 years of disease-free survival (DFS) in the training cohort. The ATG signature by the shortest distance between the ROC curve and the point at 100% true positive rate and a 0% false-positive rate was deemed as the cutoff value.

2.3 Validation of ATG signature

C-index was employed in the training, independent validation, and meta-validation cohorts respectively to assess the predictive capability of the model. For further validation, the prognostic value of the ATG signature was evaluated in CRC patients with early stages (stage I&II) and all stages in different cohorts through survival analysis. Univariate and multivariate analyses of the ATG signature and available clinical parameters was performed to assess whether the ATG signature is an independent risk factor.

2.4 Functional analysis

Enrichment of potential pathways of the ATG signature by gene ontology (GO) analysis was performed on gProfiler (<https://biit.cs.ut.ee/gprofiler/>), and gene set enrichment analysis (GSEA)[12] was conducted using the Bioconductor package 'fgsea'. Gene sets of cancer hallmarks from MSigDB with statistical significance (FDR-adjusted P<.05) were selected[13]. CIBERSORT[14] was used to dissect immune cell infiltration in different risk groups.

2.5 Statistical analysis

All statistical analyses were performed in R software (version 3.5.1). Categorical variables were reported as count. Continuous data were reported as mean with standard deviation (SD) and compared with the student's t-test. The LASSO regression was plotted using the 'glmnet' R package (version: 2.0-16). Time-dependent ROC curve analysis was done by the R package 'survivalROC' (version: 1.0.3). Survival analysis was conducted using the Kaplan–Meier method and compared with the log-rank test. Univariate and multivariate analysis of ATG signature and clinical parameters were performed using the log-rank test. The statistical significance level was set at $\alpha=0.05$, two-sided.

3 Results

3.1 ATG signature establishment

After filtration with MAD >0.5, 617 genes measured on all platforms were selected for this study. By 1000 times of random Cox univariate regressions, 58 ATGs were identified to be strongly relevant to DFS and considered as candidates for the signature (Figure 1). A LASSO Cox regression in stage I and II patients in the training cohort (Table 1) revealed 10 ATGs for the risk model (Figure 2), with coefficient of each ATG listed in Table 2. The risk model was formulated as follow: Autophagy-related risk score = $0.040346631 \times \text{exp (mRNA expression level of CD163L1)} + 0.040346631 \times \text{exp (mRNA expression level of FAM13B)} + 0.160103165 \times \text{exp (mRNA expression level of HDAC6)} + 0.05063732 \times \text{exp (mRNA expression level of HPR)} - 0.012205947 \times \text{exp (mRNA expression level of NR2C2)} - 0.027104325 \times \text{exp (mRNA expression level of NR2C2)}$

expression level of RAB12) + 0.055095935× exp (mRNA expression level of SIRT2) – 0.084226324× exp (mRNA expression level of TBC1D14) – 0.012613054× exp (mRNA expression level of TLK2) – 0.001301898× exp (mRNA expression level of TBC1D12).

Based on the time-dependent ROC curve analysis at 5 year-DFS in the training cohort, the optimal cutoff of ATG signature that divided the patients into high and low autophagy risk groups was defined as -0.009 (Figure 3). The risk scores of all patients are shown in Supplementary Table 1. Survival analysis showed the DFS rate was higher in the low autophagy risk group compared to the high autophagy risk group for patients with early stages (stage I and II) in the training cohort (GSE39582) (Figure 4A, 4B, 4C, HR, 2.76[1.56–4.82]; $P=2.06\times 10^{-4}$). So was indicated for patients in all stages in the GSE39582 dataset (Figure 5A, 5B, 5C, HR, 1.7[1.25–2.31]; $P=5.21\times 10^{-4}$).

Table 1
Characteristics of training (GSE39582), validation (TCGA) and meta-validation cohorts

	GSE39582 (training)	TCGA (validation)	Meta-validation
Number of patients	566	624	420
Patients with survival data	557	509	356
Mean age, years	66.85±13.29	66.27±12.76	66.66±12.60
Gender, n			
Male	310	332	233
Female	256	292	187
TNM stage, n			
Stage I	33	105	44
Stage II	264	230	167
Stage III	205	180	148
Stage IV	60	88	61
NA	4	21	-
CMS system, n			
CMS1	91	68	74
CMS2	232	207	168
CMS3	69	64	69
CMS4	127	117	97
NA	47	168	12
Tumor location			
Left	342	354	233
Right	224	270	185
NA	-	-	2
RFS event, n			
Yes	177	100	87
No	380	416	269
NA	9	108	64
OS event, n			
Yes	191	67	NA
No	371	557	NA
NA	4	-	420
DFS event, n			
Yes	248	146	87
No	314	386	269
NA	4	92	64
MMR status, n			

MSI	75	189	-
MSS	444	431	-
NA	47	4	420
CIMP status, n			
Positive	91	-	-
Negative	405	-	-
NA	70	624	420
CIN status, n			
Positive	353	-	-
Negative	110	-	-
NA	103	624	420
TP53 status, n			
Wild type	161	-	-
Mutation	190	-	-
NA	215	624	420
KRAS status, n			
Wild type	328	34	-
Mutation	217	30	-
NA	21	560	420
BRAF status, n			
Wild type	461	32	-
Mutation	51	3	-
NA	54	589	420
Chemotherapy adjuvant, n			
Yes	233	231	118
No	316	393	171
NA	17	-	131
RFS, relapse-free survival. OS, overall survival. DFS, disease-free survival. MMR, mismatch repair. CIMP, CpG island methylator phenotype. CIN, chromosomal instability. NA, not available.			

Table 2
Model information.

Gene	Name	Frequency in resampling	Average P-value	Coefficient
CD163L1	CD163 molecule like 1	674	0.050594905	0.040346631
FAM13B	family with sequence similarity 13 member B	625	0.050594905	0.040346631
HDAC6	histone deacetylase 6	719	0.042438619	0.160103165
HPR	haptoglobin-related protein	769	0.051952291	0.05063732
NR2C2	nuclear receptor subfamily 2 group C member 2	898	0.019300065	-0.012205947
RAB12	RAB12, member RAS oncogene family	866	0.025407812	-0.027104325
SIRT2	sirtuin 2	877	0.023493248	0.055095935
TBC1D14	TBC1 domain family member 14	998	0.002105817	-0.084226324
TLK2	tousled like kinase 2	743	0.040418795	-0.012613054
TBC1D12	TBC1 domain family member 12	999	0.002072649	-0.001301898

3.2 Validation of the ATG signature

To assess the predictive capability of the risk model, C-index was first applied to various cohorts which turned out to be 0.74 (95%CI, 0.63 - 0.85) in the GSE39582 cohort, 0.70 (95%CI, 0.54 - 0.85) in the TCGA cohort and 0.70 (95%CI, 0.51 - 0.89) in the meta-validation cohort (Table 3), higher than those of Oncotype DX colon. To further verify whether the ATG signature predicted well in various populations, we employed the same formula to the independent validation cohort (TCGA) and the meta-validation cohort (GSE37892 and GSE14333). Patients were significantly stratified into different risk groups by the ATG signature considering DFS. For early stages, CRC patients in high autophagy risk group displayed poorer DFS in both the independent validation cohort (Figure 4D, 4E, 4F, HR, 2.29[1.15–4.55]; $P=1.5\times10^{-2}$) and the meta-validation cohort (Figure 4G, 4H, 4I, HR, 2.5[1.03–6.06]; $P=3.63\times10^{-2}$). So was indicated for patients with all stages in both the independent validation (Figure 5D, 5E, 5F, HR, 1.79[1.16–2.7]; $P=5.3\times10^{-3}$) and the meta-validation cohorts (Figure 5G, 5H, 5I, HR, 1.64[1.04–2.52]; $P=3.16\times10^{-2}$). Besides, univariate and multivariate analysis further proved ATG as an independent prognostic factor after adjusting for clinical parameters such as sex and tumor stage (Table 4).

Table 3
C-index for autophagy risk compared with Oncotype DX colon in three cohorts

Cohorts	Autophagy Risk		Oncotype DX colon	
	C-index	95% CI	C-index	95% CI
GSE39582 (training)	0.74	0.63 - 0.85	0.65	0.53 - 0.77
TCGA (validation)	0.70	0.54 - 0.85	0.61	0.44 - 0.77
Meta-validation	0.70	0.51 - 0.89	0.62	0.43 - 0.82
TCGA, the Cancer Genome Atlas.				

Table 4
Univariate and multivariate analysis of ATG signature, clinical and pathologic factors in validation cohorts

Characteristic	GSE39582 (training)				TCGA(validation)				Meta-validation			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
ATG signature	2.76 (1.58 - 4.82)	0.00021	2.66 (1.52 - 4.65)	0.0006	2.27 (1.14 - 4.51)	0.016	2.27 (1.14 - 4.51)	0.016	2.50 (1.03 - 6.08)	0.036	2.50 (1.03 - 6.08)	0.036
Sex	1.51 (0.87 - 2.61)	0.14			1.29 (0.65 - 2.53)	0.46			0.86 (0.38 - 1.96)	0.73		
Age	1.01 (0.99 - 1.03)	0.4			1.01 (0.98 - 1.04)	0.46			0.97 (0.94 - 1.00)	0.08		
Tumor location	1.09 (0.63 - 1.88)	0.75			0.94 (0.48 - 1.83)	0.85			1.28 (0.55 - 2.96)	0.56		
TNM stage	7.61 (1.07 - 53.86)	0.016	7.19 (1.01 - 51.32)	0.049	1.74 (0.72 - 4.20)	0.22			2.67 (0.63 - 11.38)	0.17		
MMR status	1.60 (0.68 - 3.75)	0.28			0.78 (0.40 - 1.54)	0.47						
CIMP positive	0.99 (0.46 - 2.11)	0.97										
CIN positive	1.60 (0.71 - 3.62)	0.26										
TP53 mutation	1.40 (0.77 - 2.54)	0.26										
KRAS mutation	1.26 (0.74 - 2.13)	0.4			0.82 (0.17 - 4.07)	0.81						

ATG, autophagy-related gene. MMR, mismatch repair. CIMP, CpG island methylator phenotype. CIN, chromosomal instability. HR, hazard ratio. CI, confident interval.

3.3 Functional analysis of the ATG signature

GO analysis and GSEA were carried out to explore the biological function and signaling pathways of genes from the ATG signature. GO analysis revealed that the genes within the ATG signature were mostly involved in the regulation of autophagy and catabolic processes (Figure 6A and Supplementary Table 2). GSEA was performed between different risk groups to further investigate the pathways that were significantly affected. We found a significant enrichment in multiple immune/ inflammatory pathways in the high autophagy risk group, including the IL6/JAK/STAT3 signaling pathway, the IL2/STAT5 pathway, the IFN- α pathway, the IFN- γ pathway, the inflammatory response

pathway (Figure 6B, P value <0.005). Some cell cycle/ metabolism associated pathways, including the G2-M, oxidative phosphorylation, E2F, MYC were also significantly enriched in the high autophagy risk group, in addition to a few classic pathways like mTORC1 and epithelial-mesenchymal transmission (EMT; P value <0.005).

As there was a significant enrichment in the immune/ inflammatory pathway through GSEA analysis, we conducted immune infiltration analysis. ESTIMATE algorithm displayed significant differences in the immune score ($P=0.02$) and ESTIMATE score ($P=0.027$) between high and low autophagy risk groups in the TCGA CRC cohort (Figure 7A). Infiltration of plasma cells and Tregs were enriched significantly in the high autophagy risk group compared with the low autophagy risk group in the GSE39582 and TCGA cohort (Figure 7C). By contrast, M1 macrophage infiltration turned out to be significantly lower in the high autophagy risk group (Figure 7C).

3.4 Adjuvant chemotherapy effects on different autophagy risk groups

Survival analysis conducted for different autophagy risk groups with and without adjuvant chemotherapy respectively, showed that for early stage CRC patients without adjuvant chemotherapy in the high autophagy risk group displayed poorer DFS in the GSE39582 cohort (Figure 8A, 5[2.38–10.5]; $P=2.42\times10^{-6}$). However, DFS showed no significant difference between high and low autophagy risk groups for early CRC patients with adjuvant chemotherapy (Figure 8B, $P=4.46\times10^{-1}$). Similar results were observed in the TCGA cohort (Figure 8C, 2.05[0.9–4.67]; $P=8.22\times10^{-2}$ and Figure 8D, $P=5.84\times10^{-1}$).

4 Discussion

Thanks to the improved awareness of cancer screening, CRC is now detected at an early stage, resulting in a better rate of survival. Surgery without chemotherapy, which was deemed as the curative treatment, was carried out on the majority of patients with stage I/II colon cancer and in some cases of stage I/II rectal cancer[2]. Indeed, it enabled prevention from unnecessary side effects of chemotherapy. Nevertheless, more than 20% of patients with stage I/II CRC who underwent surgical resection still suffered recurrence[13]. Quite a few multigene prognostic signatures have been developed for CRC, but none of them graduated to widespread application due to the uncertainty of prognostic accuracy. Accordingly, an effective prognostic model composed of multiple biomarkers to distinguish early-stage patients with a high risk of recurrence is crucial and necessary for elective adjuvant chemotherapy or other targeted treatments.

Emerging studies have revealed that autophagy functions diversely in the development, maintenance, and progression of tumors. While autophagy may prevent cellular cancerous transformation in normal tissue, it acts as a survival mechanism in established tumors, especially under stress conditions and in response to chemotherapy[14]. Several autophagy inhibitors and activators have been brought up as improved chemotherapeutic options for cancer treatment[15], but without sufficient clinically significant results, especially in CRC. Accordingly, further investigation on the biological mechanism of autophagy in the tumor microenvironment deserves attention, and more targets associated with autophagy awaits to be found.

In our study, we developed a prognostic model comprised of 10 ATGs for stage I/II CRC. This ATG signature, which classified patients into high and low autophagy risk groups, demonstrated a significant difference in 5-year DFS. The C-index of the ATG signature exhibited a good clinical predicting fitness superior to Oncotype DX colon. Validation results suggested that the ATG signature could successfully predict DFS for stage I/II CRC patients after treatment. This novel model enabled us to identify CRC patients with high autophagy risk stood for increased risk of tumor recurrence. Surprisingly, despite the original intention for DFS prediction of early-stage CRC patients, the ATG signature also showed a significant effect in the prediction for all stages. Therefore, this model could be applied to predict tumor recurrence in all CRC patients independent of tumor stage. Upon the prognostic prediction, clinicians could thus make an informed decision regarding supplementary treatment regimens. For example, adjuvant chemotherapy might improve the outcome of CRC patients with high autophagy risk, for there was no significant difference in DFS between the two autophagy risk groups after adjuvant chemotherapy in this study. It might reduce the impact of autophagy on tumor relapse through massive, indiscriminate cell killing, and immunosuppression.

As we looked over the genes within the ATG signature, some of them have been found correlated to CRC, but mostly bear context-dependent biological functions in cancers, similar to autophagy. For example, the cytosolic histone deacetylase 6 (HDAC6) served as a tumor suppressor in hepatocellular carcinogenesis[16], while another study revealed HDAC6 inhibitor significantly suppressed the proliferation and viability, and induced apoptosis in CRC cells, where autophagy activation was observed[17]. Elevated Sirtuin 2 (SIRT2) was found to be associated with poor prognosis in CRC patients via its participation in tumor angiogenesis[18]. Meanwhile, in a separate study SIRT2 was found to be downregulated in colon cancer biopsies compared to adjacent noncancerous tissues, and overexpression of SIRT2 inhibited the proliferation and metastatic progression of SW480 cells[19]. In terms of autophagy-related functions, a previous

investigation reported that in response to oxidative stress or serum starvation, SIRT2 dissociated as acetylated FOXO1, which later bound to autophagy protein 7 (ATG7) and induced autophagy in tumors[20]. As these inconsistencies make it difficult to clarify the role of autophagy in CRC, we further investigated the biological functions of the ATG signature, expecting to find some clues in the autophagy-related functions in tumors.

GSEA revealed that the ATG signature included genes that were robustly involved in multiple immune/ inflammatory pathways including IL6/JAK/STAT3, IL2/STAT5, IFN- α , IFN- γ , TNF- α /NF- κ B, and the inflammatory response presented a particular relation to CRC proliferation or prognosis as previous studies revealed[21-25]. As our findings suggested that a high autophagy risk score correlated with the down-regulation of these immune/ inflammatory pathways, we speculated that autophagy might play a role in CRC tumorigenesis and tumor proliferation via an anti-immune/ anti-inflammatory response. Moreover, increased infiltration of Tregs and decreased infiltration of M1 macrophages observed in the high autophagy risk group during immune infiltration analysis seemingly catered to the anti-immune/ anti-inflammatory response. No significant difference in cytotoxic T lymphocyte was found in the immune infiltration analysis. Tregs are known to suppress both antibody-mediated and cell-mediated immune responses[26]. The pro-inflammatory M1 macrophages, a phenotype of tumor-associated macrophages (TAMs), correlated to a better prognosis in CRC patients for its tumor-suppressing function[27, 28]. By triggering an anti-immune or anti-inflammatory response, autophagy might promote polarization or re-polarization towards the M2 phenotype spontaneously and thus lead to the decrease of M1 infiltration observed. Previous studies have described the link between autophagy and macrophage polarization in tumor microenvironment. For example, mTOR which functions as a conserved kinase protein in the regulation of autophagy also participates in the polarization of monocytes into TAMs[29]. More new strategies targeting TAM polarization as well as autophagy await exploration and further studies are needed to clarify the above speculations.

However, our prognostic ATG signature relies on the gene expression profiles from microarray platforms, which make it too expensive and time-consuming to popularize in clinical application. In addition to the dataset limitations from retrospective studies, further prospective clinical tests are recommended to validate our results. Despite the limitations, our research proposes a novel perspective to predicting the prognosis of early CRC patients and offers valuable insights into the relationship between autophagy, immune/ inflammatory response, and tumorigenesis.

5 Conclusion

In conclusion, our study established a prognostic ATG signature that can effectively predict DFS for early-stage CRC patients. Meanwhile, we also revealed the possible correlation between ATG signature and immune/ inflammatory pathways.

6 Abbreviations

CRC, colorectal cancer; ATG, autophagy-related gene; DFS, disease-free survival; TCGA, The Cancer Genome Atlas; TAM, tumor-associated macrophage; Tregs, T regulatory cells; LASSO, the least absolute shrinkage and selection operator; ROC, receiver operating characteristic; GO, gene ontology.

7 Declarations

7.1 Ethics approval and consent to participate

Not applicable.

7.2 Consent for publication

Not applicable.

7.3 Availability of data and materials

The datasets analyzed for this study can be found in the Gene Expression Omnibus (GEO <http://www.ncbi.nlm.nih.gov/geo/>) and Firehose Broad GDAC portal (<http://gdac.broadinstitute.org/>).

7.4 Competing interests

The authors declare that they have no competing interests.

7.5 Funding

This study was supported by grants from National Key Clinical Discipline, the Fundamental Research Funds for the young teacher training program of Sun Yat-sen University (grant number 18ykpy02).

7.6 Authors' contributions

YZ, YR and XL contributed the conception and design of the study; YZ, SQ and XL collected the datasets; YZ, QW, SQ and XC performed the statistical analysis; YZ, QW, SQ, XC, JC, YT, JW, YR and XL participated in the data interpretation; QW wrote the first draft of the manuscript; YZ, JC, YR, XL wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

7.7 Acknowledgements

Not applicable.

8 References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA: A Cancer Journal for Clinicians* 2018, **68**(6):394-424.
2. Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL, Siegel RL: **Cancer treatment and survivorship statistics, 2019.** *CA: A Cancer Journal for Clinicians* 2019, **69**(5):363-385.
3. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB: **Colorectal cancer.** *The Lancet* 2019, **394**(10207):1467-1480.
4. Van Der Stok EP, Spaander MCW, Grünhagen DJ, Verhoef C, Kuipers EJ: **Surveillance after curative treatment for colorectal cancer.** *NAT REV CLIN ONCOL* 2017, **14**(5):297-315.
5. Brenner H, Kloor M, Pox CP: **Colorectal cancer.** *The Lancet* 2014, **383**(9927):1490-1502.
6. Rosich L, Colomer D, Roué G: **Autophagy controls everolimus (RAD001) activity in mantle cell lymphoma.** *AUTOPHAGY* 2013, **9**(1):115-117.
7. Kongara S, Karantza V: **The interplay between autophagy and ROS in tumorigenesis.** *FRONT ONCOL* 2012, **2**.
8. Carroll RG, Martin SJ: **Autophagy in multiple myeloma: What makes you stronger can also kill you.** *CANCER CELL* 2013, **23**(4):425-426.
9. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES *et al*: **Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.** *P NATL ACAD SCI USA* 2005, **102**(43):15545-15550.
10. Liberzon A, Subramanian A, Pinchback R, Thorvaldsdottir H, Tamayo P, Mesirov JP: **Molecular signatures database (MSigDB) 3.0.** *BIOINFORMATICS* 2011, **27**(12):1739-1740.
11. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov J, Tamayo P: **The Molecular Signatures Database Hallmark Gene Set Collection.** *CELL SYST* 2015, **1**(6):417-425.
12. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA: **Robust enumeration of cell subsets from tissue expression profiles.** *NAT METHODS* 2015, **12**(5):453-457.
13. Markle B, May EJ, Majumdar APN: **Do nutraceuticals play a role in the prevention and treatment of colorectal cancer?** *CANCER METAST REV* 2010, **29**(3):395-404.
14. Mokarram P, Albokashy M, Zarghooni M, Moosavi MA, Sepehri Z, Chen QM, Hudecki A, Sargazi A, Alizadeh J, Moghadam AR *et al*: **New frontiers in the treatment of colorectal cancer: Autophagy and the unfolded protein response as promising targets.** *AUTOPHAGY* 2017, **13**(5):781-819.
15. Koustas E, Sarantis P, Kyriakopoulou G, Papavassiliou AG, Karamouzis MV: **The interplay of autophagy and tumor microenvironment in colorectal cancer—ways of enhancing immunotherapy action.** *CANCERS* 2019, **11**(4).

16. Yang HD, Kim HS, Kim SY, Na MJ, Yang G, Eun JW, Wang HJ, Cheong JY, Park WS, Nam SW: **HDAC6 Suppresses Let-7i-5p to Elicit TSP1/CD47-Mediated Anti-Tumorigenesis and Phagocytosis of Hepatocellular Carcinoma.** *HEPATOLOGY* 2019, **70**(4):1262-1279.
17. Chen M, Lin Y, Liao Y, Liou J, Chen C: **MPT0G612, a Novel HDAC6 Inhibitor, Induces Apoptosis and Suppresses IFN-gamma-Induced Programmed Death-Ligand 1 in Human Colorectal Carcinoma Cells.** *CANCERS* 2019, **11**(161710).
18. Hu F, Sun X, Li G, Wu Q, Chen Y, Yang X, Luo X, Hu J, Wang G: **Inhibition of SIRT2 limits tumour angiogenesis via inactivation of the STAT3/VEGFA signalling pathway.** *Cell Death and Disease* 2019, **10**(1).
19. Zhang LL, Zhan L, Jin YD, Min ZL, Wei C, Wang Q, Chen YJ, Wu QM, Hu XM, Yuan Q: **SIRT2 mediated antitumor effects of shikonin on metastatic colorectal cancer.** *EUR J PHARMACOL* 2017, **797**:1-8.
20. Zhao Y, Yang J, Liao W, Liu X, Zhang H, Wang S, Wang D, Feng J, Yu L, Zhu WG: **Cytosolic FoxO1 is essential for the induction of autophagy and tumour suppressor activity.** *NAT CELL BIOL* 2010, **12**(7):665-675.
21. Nichols PH, Ward U, Ramsden CW, Primrose JN: **The effect of 5-fluorouracil and alpha interferon and 5-fluorouracil and leucovorin on cellular anti-tumour immune responses in patients with advanced colorectal cancer.** *BRIT J CANCER* 1994, **70**(5):946-949.
22. Eguchi J, Hiroishi K, Ishii S, Mitamura K: **Interferon-alpha and interleukin-12 gene therapy of cancer: interferon-alpha induces tumor-specific immune responses while interleukin-12 stimulates non-specific killing.** *CANCER IMMUNOL IMMUN* 2003, **52**(6):378-386.
23. De Simone V, Franzè E, Ronchetti G, Colantoni A, Fantini MC, Di Fusco D, Sica GS, Sileri P, MacDonald TT, Pallone F *et al*: **Th17-type cytokines, IL-6 and TNF- α synergistically activate STAT3 and NF- κ B to promote colorectal cancer cell growth.** *ONCOGENE* 2015, **34**(27):3493-3503.
24. Park JH, Van Wyk H, McMillan DC, Quinn J, Clark J, Roxburgh CSD, Horgan PG, Edwards J: **Signal transduction and activator of transcription-3 (STAT3) in patients with colorectal cancer: Associations with the phenotypic features of the tumor and host.** *CLIN CANCER RES* 2017, **23**(7):1698-1709.
25. Giordano G, Parcesepe P, D'Andrea MR, Coppola L, Di Raimo T, Remo A, Manfrin E, Fiorini C, Scarpa A, Amoreo CA *et al*: **JAK/Stat5-mediated subtype-specific lymphocyte antigen 6 complex, locus G6D (LY6G6D) expression drives mismatch repair proficient colorectal cancer.** *Journal of Experimental and Clinical Cancer Research* 2019, **38**(1).
26. Wing K, Sakaguchi S: **Regulatory T cells exert checks and balances on self tolerance and autoimmunity.** *NAT IMMUNOL* 2010, **11**(1):7-13.
27. Edin S, Wikberg ML, Dahlin AM, Rutegård J, Öberg Å, Oldenberg PA, Palmqvist R: **The Distribution of Macrophages with a M1 or M2 Phenotype in Relation to Prognosis and the Molecular Characteristics of Colorectal Cancer.** *PLOS ONE* 2012, **7**(10).
28. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaili SA, Mardani F, Seifi B, Mohammadi A, Afshari JT, Sahebkar A: **Macrophage plasticity, polarization, and function in health and disease.** *J CELL PHYSIOL* 2018, **233**(9):6425-6440.
29. Chen P, Cescon M, Bonaldo P: **Autophagy-mediated regulation of macrophages and its applications for cancer.** *AUTOPHAGY* 2014, **10**(2):192-200.

Figures

Figure 1

Flow chart

Figure 2

Discovered ATG signature for prognostic prediction of colorectal cancer. (A). Ten ATGs signature found from the LASSO Cox regression. (B). Heatmap of the identified 10-gene signature.

Figure 3

Time-dependent ROC curve for 5-year disease-free survival in stage I/II CRC patients in training cohort.

Figure 4

The association of the ATG signature with DFS in early stage patients with CRC. Patients with CRC of early stage were ranked by autophagy risk scores in the training cohort (A), the TCGA cohort (D) and the meta-validation cohort (G). Time-dependent ROC curves for DFS in early stage (stage I&II) patients achieved with different duration in the training cohort (B), the TCGA cohort (E) and the meta-validation cohort (H). Kaplan–Meier curves showed DFS of early stage patients in low and high autophagy groups in training cohort(C), the TCGA cohort (F) and meta-validation cohort (I), respectively. P values comparing risk groups were calculated with the log-rank test. Hazard ratios (HRs) and 95% CIs are for low vs high autophagy risk. CRC = colorectal cancer, ATG = autophagy-related gene, DFS = disease-free survival.

Figure 5

The association of the ATG signature with DFS in CRC patients with all stages. Patients with CRC of early stage were ranked by autophagy risk scores in the training cohort (A), the TCGA cohort (D) and the meta-validation cohort (G). Time-dependent ROC curves for DFS in CRC patients achieved with different duration in the training cohort (B), the TCGA cohort (E) and the meta-validation cohort (H). Kaplan–Meier curves showed DFS of CRC patients in low and high autophagy groups in training cohort (C), the TCGA cohort (F) and meta-validation cohort (I), respectively. P values comparing risk groups were calculated with the log-rank test. Hazard ratios (HRs) and 95% CIs are for low vs high autophagy risk. CRC = colorectal cancer, ATG = autophagy-related gene, DFS = disease-free survival.

Figure 6

Functional analysis of the 10 autophagy signature genes. (A). Gene ontology (GO) of the 10 autophagy signature genes. (B). GSEA showed some immune/ inflammatory pathways downregulated in high autophagy risk patients.

Figure 7

Page 17/18

Immune infiltration status of ATG signature risk groups. (A). ESTIMATE algorithm in the TCGA cohort. (B). 22 immune cells' abundance for different immune risk groups. (C). Plasma cells and T cells regulatory were enriched in the high-risk group. The macrophage M1 was enriched in the low-risk group. P-values are based on the Wilcoxon Test.

Figure 8

Effects of adjuvant chemotherapy between different autophagy risk groups in early stage CRC patients. Kaplan–Meier curves showed DFS of early stage patients between low and high autophagy groups with and without adjuvant chemotherapy respectively in the GSE39582(A and B) cohort and the TCGA cohort(C and D). P values comparing risk groups were calculated with the log-rank test. Hazard ratios (HRs) and 95% CIs are for low vs high autophagy risk. CRC = colorectal cancer, DFS = disease-free survival.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable2.xlsx](#)
- [SupplementaryTable1.xlsx](#)