Prognostic Model of Colorectal Cancer Constructed by Eight Immune-Related Genes

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Background: Colorectal cancer (CRC) is a common malignant tumor of the digestive tract with a high mortality rate. Growing evidence demonstrates that immune-related genes play a prominent role in the occurrence and development of CRC. The aim of this study was to investigate the prognostic value of immune-related genes in CRC.

Methods: Gene expression profiles and clinical data of 568 CRC and 44 non-tumorous tissues were obtained from The Cancer Genome Atlas (TCGA) database. First, we performed a differentially expressed gene (DEG) analysis and univariate Cox regression analysis to determine the DEGs associated with overall survival. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were subsequently performed for prognostic immune-related genes. Then, a multivariate Cox regression analysis was performed to establish the immune prognostic model and identify the independent prognostic factors of CRC. Next, in vitro experiments were done to further validate the model. Finally, we analyzed the correlation among immune-related genes, clinical traits, and immune cell infiltration.

Results: In total, 3,702 DEGs were obtained, and 338 prognostic immune-related genes were identified. Among them, 45 genes were significantly correlated with the prognosis of CRC patients. A TF-mediated network was set up to explore its internal mechanism. GO and KEGG analyses further illustrated that these genes were enriched in immune-and inflammatory-related pathways. Then, a prognostic prediction model composed of eight immune-related genes (SLC10A2, UTS2, FGF2, UCN, IL1RL2, ESM1, ADIPOQ, and VIP) was constructed. The AUC of the ROC curve for 1, 3, 5, and 10 years overall survival (OS) was 0.751, 0.707, 0.680, and 0.729, respectively. The survival analysis suggested that the OS of the high-risk group was significantly poorer than that of the low-risk group. Meanwhile, in vitro assays revealed that ESM1 and SLC10A2 exert opposing roles in colon cancer cell proliferation, validating the accuracy of the model. The correlation analysis indicated that immune cell infiltration was positively related to the model.
INTRODUCTION

Colorectal cancer (CRC) is known as the third most common cancer globally and has the second-highest leading number of cancer-related deaths worldwide (Keum and Giovannucci, 2019). Every year, an estimated 9,00,000 people die from CRC all over the world, and more than 1.8 million people are diagnosed with CRC (Dekker et al., 2019; Safiri et al., 2019). The morbidity and mortality rate of CRC has been rising in Asia (Onyoh et al., 2019), which has become a major public health problem. Because the early symptoms of CRC are not obvious, most patients are overlooked (Or et al., 2017). They are often diagnosed in intermediate or late stages and have poor prognoses with the symptoms of hematochezia, abdominal pain, and altered bowel habits, as well as emaciation (Dekker et al., 2019). Despite recent progress in testing and treatments, the overall prognosis for patients with CRC remains poor because biomarkers for early detection and prognosis prediction are lacking (Keum and Giovannucci, 2019). Thus, it is essential to explore valuable diagnostic and prognostic factors, as well as potential effective therapeutic targets.

The immune system is important for the progression of cancer (Patel and Minn, 2018). Immunotherapy is a new kind of therapy for cancer and other chronic conditions that targets the human immune system (Thyer et al., 2013; Zhang et al., 2019). It is commonly accepted that CRC is a clinically heterogeneous and immunogenic disease (Leman et al., 2018). There are various therapeutic approaches for CRC, such as surgery, radiotherapy, and chemotherapy (Dekker et al., 2019). Furthermore, targeted therapy and immunotherapy are more conducive to the treatment of CRC patients (Ganesh et al., 2019). Immune cells play a key role; from tumor growth to their invasion and metastasis (Ciardiello et al., 2019). An increasing number of studies have found that a large number of immune-related genes are associated with the development of CRC (Ceredia et al., 2016; Yu et al., 2019). In recent studies, prognostic-related genes associated with CRC patients were extracted according to their gene expression profile (Sun et al., 2018; Zuo et al., 2019), while Bai et al. (2020) obtained immune-related genes with the prognosis in CRC patients. Notwithstanding, there are currently no immune-related differentially expressed genes (DEGs) to systematically evaluate the prognosis of CRC.

Conclusions: This study screened prognosis-related immune genes and developed a prognostic prediction model of CRC. These findings may help provide potential novel prognostic biomarkers and therapeutic targets for CRC. At the same time, the understanding of the CRC immune microenvironment status was deepened.

Keywords: colorectal cancer, immune-related gene, prognostic model, bioinformatics, immune microenvironment

MATERIALS AND METHODS

Data Collection

The study scheme is illustrated in Figure 1. Gene expression matrix data of CRC samples were obtained from The Cancer Genome Atlas (TCGA) data portal1 (up to February 26, 2020). Transcriptome information for cancers with “colon” or “rectum” as the primary site from a “TCGA-COAD” and “TCGA-READ” project were included. HT-Seq FPKM data of 568 CRC and 44 non-tumorous tissues were downloaded for further analysis. From the database, we also searched the clinical information of the related patients, including survival information, age, gender, tumor stage, and histology classification. A total of 2,498 immunologically relevant genes were retrieved from the Immunology Database and Analysis Portal (ImmPort)2 database (Bhattacharya et al., 2018). For exploring the regulatory mechanism of immune genes related to CRC, we downloaded 318 transcription factors (TFs) via Cistrome Cancer3 (Mei et al., 2017), which is a comprehensive resource for genetic research in cancers.

Differentially Expressed Gene (DEG) Identification

From TCGA we obtained the expression matrix data of 13,673 RNAs in CRC. The DEGs between CRC and adjacent normal tissues were identified using the Wilcoxon test method with the “limma” (Ritchie et al., 2015) package in R software (version 3.6.2). Meanwhile, immune-related DEGs and TFs in CRC were screened, with the conditions of | LogFC | > 1 and a false discovery rate (FDR) of < 0.05 was set as significant.

Abbreviations: CRC, colorectal cancer; TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes; TFs, transcription factor; TIMER, Tumor immune estimation resource; GO, gene ontology database; KEGG, The Kyoto Encyclopedia of Genes and Genomes; ROC, receiver operating characteristic; AUC, area under the ROC curve; OS, overall survival.

1https://portal.gdc.cancer.gov/
2https://www.immport.org/
3http://cistrome.org/
The upregulated and downregulated genes were visualized on volcano plots, while heatmaps were generated to show the expression profiles of significant immune-related DEGs and TFs using the "pheatmap" package. To identify the biological properties of immune-related DEGs, GO, and KEGG pathway enrichment analyses were conducted using the “clusterProfiler,” “enrichplot,” and “ggplot2” packages. Enrichment results with an FDR (false discovery rate) of < 0.05 were recognized as significant functional categories.

**Identification of Prognostic Immune-Related Genes and Construction of the Network With TFs**

To make the results more accurate, patients with a follow-up time of more than 90 days were included in the survival analyses. Univariate Cox regression was applied to examine the prognostic value of immune-related DEGs in CRC patients using the “survival” package. \( P \)-values of < 0.05 were considered as significant and shown by a forest plot. Genes with a hazard ratio (HR) > 1 were identified as high-risk genes, while a HR < 1 was identified as low risk. The potential biological functions of these prognostic immune-related DEGs were further performed by GO and KEGG enrichment analyses. To determine the mechanisms between the survival-related immune genes and transcription factors, a Pearson’s correlation test was conducted in the R software. Both the correlation coefficient (cor) and \( P \)-value were estimated. The study set the filtering criteria as \( |\text{cor}| > 0.4 \) and \( P < 0.001 \). It was considered that cor > 0 showed a relationship of positive correlation, while cor < 0 indicated a negative correlation. The regulatory network between TFs and prognostic immune-related genes was constructed and visualized via Cytoscape 3.7.1.

**Construction of the Prognostic Immune-Related Gene Signature for CRC**

After acquiring the prognostic immune-related genes of CRC with a \( P \)-value of < 0.01, a multivariate Cox regression analysis was implemented to develop a prognostic model using the “survival” package. The model was utilized to evaluate the
connection of overall survival (OS) and immune-related genes. According to the model, risk scores were calculated, and patients were divided into high and low risk groups on the basis of the median risk score. For validation of the model, a Kaplan-Meier log-rank analysis was implemented via the “survminer” and “survival” packages. Then, the receiver operating characteristic (ROC) curve was generated using the “surv ROC” package, as well as a risk plot performed using the “pheatmap” package.

### Independent Prognostic Analysis and Clinical Correlation Analysis
Univariate and multivariate independent prognostic analyses were utilized by the “survival” package. The aim was to evaluate whether the risk score is an independent prognostic factor that is associated with overall survival. A clinical relevance analysis was conducted using the “beeswarm” package to assess the correlation between prognostic immune-related DEGs and clinical traits.

### Correlation Analysis Between Risk Score and Immune Cell Infiltration
To examine the connection between the immune-related signature and immune cell infiltration, we obtained the matrix of immune cells from the Tumor Immune Estimation Resource (TIMER)\(^4\), which is a useful web server for the systematic analysis of immune infiltrates in cancer. Users can predict the abundancies and proportion of six immune cell subsets (B cells, CD4\(^+\) T cells, CD8\(^+\) T cells, dendritic cells, macrophages, and neutrophils). A Pearson’s correlation test was utilized in R to explore the correlation between the risk scores and tumor infiltrating immune cells.

### Cell Lines and Cell Transfection
Human CRC cell lines HCT-116 were cultured in RPMI-1640 medium, while SW-480 cell lines were cultured in DMEM medium. Both media contained 10\(\%\) fetal bovine serum (FBS; Gibco, United States), 100 U/mL penicillin, 100 \(\mu\)g/mL streptomycin. ESM1-specific siRNA, SLC10A2-specific siRNA, and control siRNA were purchased from the Generay Biotech (Shanghai) Co., Ltd. The siRNA sequence targeting ESM1 were as follows: sense siRNA:\(5’\)-CUC UCA CGG AGC AUG ACA UTT-3’; and antisense siRNA: \(5’\)-AUG UCA UGA UCC GUG AGA GTT-3’. The targeted sequences of SLC10A2 were sense siRNA: \(5’\)-CCA AAG CGC GAG CAU UTT-3’; and antisense siRNA: \(5’\)-AUU GAU CCA GGC GCU UUG GTT-3’. ESM1 and SLC10A2 siRNA transfection was performed with Lipofectamine 2000 (Invitrogen) in 6-well plates following the manufacturer’s protocol.

### Cell Proliferation Assay
The cell proliferation and viability assays were assessed by Cell Counting Kit-8 (CCK-8, Biosharp, China). Cells were collected at 24 h after transfection and were inoculated into 96-well culture plates (2 \(\times\) 10\(^3\) cells/well). Subsequently, 10 \(\mu\)L of CCK-8 was added at different time points to each well and incubated at 37\(^\circ\)C for 2 h. The optical density (OD) was measured at 450 nm with a microplate reader (Thermo Fisher Scientific, Waltham, MA, United States) and the cell viability was calculated.

### Statistical Analysis
All analyses were conducted using R version 3.6.2 and GraphPad Prism 8.0 (GraphPad Software, San Diego, United States). Significance was determined as \(*p < 0.05\), \(**p < 0.01\) unless otherwise specified.

### RESULTS

#### Differentially Expressed Immune-Related Genes in CRC
A total of 3,702 genes (2,196 upregulated and 1,506 downregulated) were considered as DEGs in CRC tissues compared with adjacent normal tissues. Among them, there were 338 immune-related genes (174 upregulated and 164 downregulated) that were identified as differentially expressed. The distribution of DEGs and immune-related DEGs between CRC and normal tissues was visualized by volcano plots (Figures 2A,C). The heatmaps revealed the expression changes of genes and immune-related genes, which can be obviously distinguished between CRC and a normal sample (Figures 2B,D).

All 338 immune-related DEGs were analyzed by GO and KEGG analyses. The results indicated that the most enriched biological processes (BP) were leukocyte migration, cell chemotaxis, and leukocyte chemotaxis. For cellular components (CC), immune-related DEGs were mainly involved in the external side of the plasma membrane, collagen-containing extracellular matrix, and cytoplasmic vesicle lumen. The major functional categories in molecular function (MF) were receptor ligand activity, cytokine activity, and cytokine receptor binding (Figure 3A). The KEGG pathway enrichment analysis revealed that immune-related genes were particularly enriched in cytokine–cytokine receptor interaction and neuroactive ligand–receptor interaction, as well as in viral protein interaction with cytokines and cytokine receptors (Figure 3B).

#### Screening of Prognostic Immune-Related DEGs in CRC
A univariate Cox analysis was performed on 338 immune-related differential genes in 471 CRC samples, and 45 genes related to overall survival were obtained (Figure 4 and Supplementary Table 1). Among them, 35 genes were identified as high-risk genes, while 10 were low-risk genes. The GO analysis revealed that these prognostic genes were mainly involved in positive chemotaxis, cell chemotaxis, and the regulation of chemotaxis (Figures 5A,B). The most enriched KEGG pathways were cytokine–cytokine receptor interaction, neuroactive ligand–receptor interaction, and apoptosis–multiple species (Figures 5C,D). All these pathways were significantly related to the development of CRC.

\(^4\)https://cistrome.shinyapps.io/timer/
FIGURE 2 | DEGs and immune-related DEGs in CRC. (A) Volcano plot of DEGs. The red dots represent the upregulated genes and the green dots represent the downregulated genes in CRC. Black dots represent no statistical significance. (B) Heatmap of DEGs. The process from low to high expression of each gene is shown by the color from green to red. (C) Volcano plot of immune-related DEGs. The orange dots indicate the upregulated genes and the blue dots indicate the downregulated immune-related genes in CRC. (D) Heatmap of immune-related DEGs. Orange represents higher expression while blue represents lower expression in CRC.

TF Regulatory Network
Our results showed that 69 TFs (45 upregulated and 24 downregulated) were differentially expressed between CRC and normal tissues. The distribution of TFs was represented by a volcano plot (Figure 6A), while the expression changes of the TFs were revealed by a heatmap (Figure 6B). We also identified 9 prognosis-related TFs using the univariate Cox regression analysis, including TCF7L1, SALL4, ELF5, SNAPC4, WWTR1, FOSL1, TEAD4, FOXM1, and NCAPG (Supplementary Table 2). Relevant TFs and immune-related genes were selected by correlation analysis, and a regulatory network was established to elucidate the mechanism of immune-related genes in CRC. The network consists of 38 nodes (20 TFs, 12 high-risk, and 6 low-risk prognosis-related genes) with 68 edges (Figure 6C). It was shown that NR3C1 regulated most of the high-risk genes. Meanwhile, all the TFs in the network had positive relationships with the immune-related genes.

Construction of Immune-Related Prognostic Model for CRC
By utilizing the univariate Cox analysis, 13 prognostic immune-related genes were filtered. A total of eight genes were finally identified to develop a prognostic model after performing six multivariate Cox analyses (Table 1). Based
on the expression levels of genes and the corresponding Cox regression coefficient, the formula was shown as: the risk score = (expr_{SLC10A2} * 0.633) + (expr_{ADIPOQ} * 0.087) + (expr_{FGF2} * 0.326) + (expr_{ESM1} * 0.106) + (expr_{UCN} * 0.283) + (expr_{UTS2} * 0.333) + (expr_{VIP} * 0.044) + (expr_{IL1RL2} * 0.221). All eight genes were high-risk genes, with HRs of > 1. The CRC patients were divided into a high-risk group (n = 235) and a low-risk group (n = 236) on the basis of the median score of 0.837 (Figure 7A). A survival status overview and eight-gene expression heatmap were established (Figures 7B,C). The Kaplan-Meier analysis
confirmed that patients in the high-risk group had poorer overall survival (OS) compared to those in the low-risk group. The 5 years survival rate of the high-risk group was 0.499 (95% CI: 0.376–0.664), while that of the low-risk group was 0.744 (95% CI: 0.630–0.880) (Figure 7D). The AUC of the ROC curve for 1, 3, 5, and 10 years OS was 0.751, 0.707, 0.680, and 0.729, respectively. The results indicate that the prognostic model has good sensitivity and specificity (Figure 7E).

Association Between Prognostic Model and Overall Survival

We obtained data from 413 CRC patients with complete clinical information, including survival status, survival time, age, gender, pathological stage, and TNM stage. As shown in Table 2, detailed clinical characteristics of the patients are listed. The results of the Chi-squared test suggest that differences in the distribution of the N classification in low-risk and high-risk groups were statistically significant. To further confirm the independent predictive power of the prognostic model, univariate and multivariate Cox regression analyses were applied. The univariate analysis showed that age (P = 0.005), pathological stage (P < 0.001), TNM classification (P < 0.001), and the immune-related prognostic model (P < 0.001) were significantly correlated with overall survival. The multivariate analysis indicated that immune-related prognostic models (P < 0.001), age (P < 0.001), and T classification (P = 0.008) remained independent prognostic factors related to OS (Table 3).
Clinical Correlation Analysis

We performed a correlation analysis of the immune-related genes involved in the prognostic model and clinical parameters. It was shown that the gene expression of ESM1, VIP, ADIPOQ, UTS2, and UCN were associated with clinical traits. Meanwhile, with the gradual increase of stage and T and N classification, the risk score of the prognostic model increased, suggesting the accuracy of the model (Figure 8). The association between the risk score and immune cell infiltration was examined. Results indicated that CD4⁺ T cells ($P < 0.001$), CD8⁺ T cells ($P = 0.03$), dendritic cells ($P = 0.009$), macrophages ($P < 0.001$), and neutrophils ($P = 0.04$) were positively related to the risk score, suggesting that the greater the risk value, the higher the immune infiltration.
FIGURE 6 | TF regulatory network. (A) Volcano plot of differentially expressed TFs. The salmon dots represent the upregulated TFs and the cyan dots represent the downregulated TFs in CRC. Black dots represent no statistical significance. (B) Heatmap of differentially expressed TFs. The progression from low to high expression of TFs is shown by the color from cyan to salmon. (C) Construction of the network with TFs and prognostic immune-related differentially expressed genes. The blue triangles, red circles, and green circles represent TFs, high-risk genes, and low-risk genes, respectively.
TABLE 1 | General characteristics of the eight immune-related genes that construct the prognostic model.

| Gene symbol | logFC | FDR     | Coef  | HR   | 95% CI    | P-value |
|-------------|-------|---------|-------|------|----------|---------|
| SLC10A2     | −7.383| 9.12E−29| 0.633 | 1.883| 1.203−2.948| 5.64E−03|
| UTS2        | 1.011 | 2.52E−02| 0.333 | 1.385| 1.155−1.661| 4.36E−04|
| FGF2        | −1.488| 2.62E−13| 0.326 | 1.385| 1.155−1.661| 4.36E−04|
| UCN         | 2.390 | 4.47E−20| 0.283 | 1.327| 1.165−1.512| 2.06E−05|
| IL1RL2      | 1.160 | 1.79E−04| 0.221 | 1.248| 1.093−1.425| 1.05E−03|
| ESM1        | 5.974 | 2.01E−25| 1.112 | 1.044| 1.184−9.47E−04|
| ADIPOQ      | −1.746| 9.04E−22| 0.086 | 1.091| 1.034−1.151| 9.47E−05|
| VIP         | −3.380| 1.97E−24| 0.044 | 1.045| 1.017−1.075| 1.68E−03|

In addition, B cells had no significant correlation with risk scores (Figure 9).

ESM1 and SLC10A2 Exert Opposing Roles in Colon Cancer Cell Proliferation

Furthermore, we attempted to validate the functional effects of ESM1 and SLC10A2 on colon cancer cells in vitro. We transfected HCT-116 and SW-480 cell lines with si-ESM1 and si-SLC10A2 or their negative controls (NC). Afterward, cell proliferation was estimated using a CCK-8 assay. Results showed that si-ESM1 inhibits the growth of tumor cells, while si-SLC10A2 promotes the proliferation of tumor cells (Figures 10A,B).

DISCUSSION

CRC remains one of the most fatal malignant tumors with a poor prognosis globally (Dekker et al., 2019; Gbd. 2017 Colorectal Cancer Collaborators, 2019). Accurate overall survival prediction of CRC is of great significance for selecting therapeutic options and improving the prognosis of patients. However, to date, no robust and effective biomarkers have been identified to accurately predict the survival of CRC patients. The importance of immunity in cancer development and progression is also well recognized (Finotello et al., 2019). Immunotherapy is increasingly important in the treatment of cancer (Helmy et al., 2013; Yang, 2015). Thus, it is necessary to construct an immune-related prediction model for the prognosis of CRC patients.

In this study, 338 immune-related DEGs were screened from 568 samples of CRC and 44 normal tissues. A GO functional analysis revealed that the mentioned genes were mainly related...
FIGURE 8 | Clinical relevance analysis. Association between the prognostic immune-related signature and clinical factors in CRC.

FIGURE 9 | Correlation analysis between risk score and abundances and proportion of six types of immune cell subsets.
We identified eight immune-related genes (SLC10A2, UTS2, SLC10A2, UTS2, UCN, IL1RL2, ESM1, ADIPOQ, and VIP) as potential biomarkers of CRC and constructed the prognostic model after a multivariate Cox regression analysis. Next, the risk score was obtained using the model. The accuracy of the model was further demonstrated through the evaluation of the survival analysis, risk analysis, ROC curve, and independent prognostic analysis. Thus, the risk score combined with these eight genes may be a viable clinical predictor of the progression and prognosis in CRC patients.

Compared with normal tissues, UTS2, UCN, IL1RL2, and ESM1 are upregulated in CRC tissues. UTS2 (Urotensin-II) is a somatostatin-like cyclic heptapeptide (Kim et al., 2017) and participates in the occurrence of different cancers, especially colon, breast, and prostate cancers (Giulianelli et al., 2019). It is extensively expressed in vascular endothelial cells and various tissues such as the small intestine, colon, brain, heart, spleen, aorta, and so on (Yumrutas et al., 2015). Several biological processes in tumor biology are regulated by UTS2, including mitogenic, cell proliferation, and angiogenesis (Federico et al., 2017). Urocortin (UCN) is mainly expressed in reproductive organs or urinary systems such as endometrium, testes, epididymis, and urothelial. UCN is a member of the CRH family, and it can inhibit the invasion and metastatic spread of endometrial cancer (Owens et al., 2017). Interleukin-1 receptor-like 2 (IL1RL2), also known as IL-36 receptor, distributed in the intestine, kidney, skin, and brain, is produced by monocytes and T/B lymphocytes. IL1RL2 plays a crucial role in the inflammatory response (Mahil et al., 2017); it is also related to the distant metastasis of breast cancer and the tumor microenvironment (Chen et al., 2019). However, the relationship of these genes with the prognosis of CRC has not been previously studied. Gene functional enrichment analyses show that these genes are significantly enriched in the vascular process in the

| Parameter          | Whole cohort (n = 413) | Low risk (n = 203) | High risk (n = 210) | p       |
|--------------------|------------------------|--------------------|--------------------|---------|
| Age at diagnosis, y| 0.489                  |                    |                    |         |
| < 60               | 180 (43.6)             | 92 (22.3)          | 88 (21.3)          |         |
| > 60               | 233 (56.4)             | 111 (26.9)         | 122 (29.5)         |         |
| Gender             | 0.489                  |                    |                    |         |
| Male               | 228 (55.2)             | 116 (28.1)         | 112 (27.1)         |         |
| Female             | 185 (44.8)             | 87 (21.1)          | 98 (23.7)          |         |
| Pathological stage | 0.071                  |                    |                    |         |
| I                  | 73 (17.7)              | 43 (10.4)          | 30 (7.3)           |         |
| II                 | 164 (39.7)             | 86 (20.8)          | 78 (18.9)          |         |
| III                | 112 (27.1)             | 47 (11.4)          | 65 (15.7)          |         |
| IV                 | 64 (15.5)              | 27 (6.5)           | 37 (9)             |         |
| T classification   | 0.14                   |                    |                    |         |
| T1                 | 12 (2.9)               | 5 (1.2)            | 7 (1.7)            |         |
| T2                 | 70 (16.9)              | 43 (10.4)          | 27 (6.5)           |         |
| T3                 | 287 (69.5)             | 136 (32.9)         | 151 (36.6)         |         |
| T4                 | 44 (10.7)              | 19 (4.6)           | 25 (6.1)           |         |
| N classification   | 0.014*                 |                    |                    |         |
| NO                | 244 (59.1)             | 134 (32.4)         | 110 (26.6)         |         |
| N1                | 102 (24.7)             | 44 (10.7)          | 58 (14)            |         |
| N2                | 67 (16.2)              | 25 (6.1)           | 42 (10.2)          |         |
| M classification   | 0.277                  |                    |                    |         |
| M0                | 349 (84.5)             | 176 (42.6)         | 173 (41.9)         |         |
| M1                | 64 (15.5)              | 27 (6.5)           | 37 (9)             |         |

*p < 0.05. †Patients with information unavailable on tumor stage (15 patients); M classification (53 patients) were excluded from the comparison.

TABLE 3 | Univariate and multivariate analyses of overall survival in colorectal carcinoma patients.

| Parameter          | Univariate analysis | Multivariate analysis |
|--------------------|---------------------|-----------------------|
|                    | HR 95% CI           | P         | HR 95% CI           | P         |
| Age                | 1.034 1.010–1.059   | 0.005     | 1.051 1.025–1.077   | 8.88E−05  |
| Gender             | 1.025 0.632–1.661   | 0.921     | 0.835 0.505–1.381   | 0.483     |
| Pathological stage | 2.557 1.931–3.384   | 5.39E−11  | 1.307 0.546–3.128   | 0.548     |
| T classification   | 3.618 2.225–5.883   | 2.17E−07  | 2.157 1.219–3.817   | 0.008     |
| N classification   | 2.254 1.696–2.996   | 2.10E−08  | 1.353 0.825–2.221   | 0.231     |
| M classification   | 5.272 3.215–8.643   | 4.39E−11  | 2.077 0.624–6.914   | 0.233     |
| Prognostic model   | 1.256 1.189–1.327   | 3.21E−16  | 1.202 1.131–1.277   | 2.56E−09  |

Bold values indicate P < 0.05. HR, hazard ratio; CI, confidence interval.
immune system, digestive system, and can be secreted by pathogenesis of various cancers (Sastry et al., 2017). VIP is may play an important role in the prediction of CRC. CRC risk (Nimptsch et al., 2017), suggesting that adiponectin adiponectin levels have been observed (Divella et al., 2019). During the development of CRC liver metastasis, decreased adiponectin and susceptibility to cancer (Divella et al., 2017). significant associations between a decreased expression level of (ADIPOQ) is an adipokine mainly secreted by adipocytes with Ras signaling pathway, PI3K-Akt signaling pathway, and Rap1 formation (Chrzanowska-Wodnicka, 2013). These results further support the idea that these pathways serve an important role in CRC, which were consistent with the results in our study.

The current study found a positive correlation between the prognostic model and immune cell infiltration. Data indicate that higher infiltration levels of CD4+ T cells, CD8+ T cells, dendritic cells, macrophages, and neutrophils may be observed in high-risk CRC patients. This demonstrates that the model can be used as an effective tool for an increased immune cell infiltration prediction. Recent research has reported that intratumoral CD4+ T cells may be a meaningful predictor of CRC (Kuwahara et al., 2019). CD8+ T cells are associated with tumor immune recognition and destruction (Sokol et al., 2015), and the activation of CD8+ T cells is increased via dendritic cells (Ziegler et al., 2018), which can be included in immunotherapy against cancer. Macrophages are associated with tumor growth, angiogenesis, and metastasis and are good potential anticancer targets (Wei et al., 2019). Neutrophils contribute to the activation of immune cells, tumor growth, and proliferation (Governa et al., 2017) and can be used as key clinical biomarkers and therapeutic targets.

Our study provides new evidence that immune-related DEGs are associated with the CRC prognosis. The results further confirm and expand the understanding that immune genes and cells regulate CRC progression. An immune-related prognostic model was constructed, providing new insights for the diagnosis and treatment targets of CRC in immunotherapy.

Despite these promising results, the limitations of this study should be taken into consideration. This study utilized retrospective transcriptome analysis data that could not completely reflect the overall immune status. Therefore, clinical data still need to be collected to validate the accuracy of the prognostic model. In addition, further experiments are needed to elucidate the relevant mechanisms of immunogenomics in the occurrence, progression, and metastasis of CRC.

**CONCLUSION**

In summary, we identified numerous immunologically DEGs that are significantly related to the prognosis of CRC. Moreover, lymphocytes, macrophages, and mast cells. Evidence has already shown that VIP can prevent the development of liver cancer through apoptosis of the cAMP/Bcl-xL pathway (Hara et al., 2019), but its prognostic significance in CRC requires further investigation.

Previous studies have shown that neuroactive ligand–receptor interaction pathways interact with the microenvironment cells and tumor cells (Lv et al., 2020), meanwhile, hypomethylation-high expression genes in CRC were also enriched in the cytokine–cytokine receptor interaction (Liu et al., 2017). The Ras signaling pathway, which is reported to be the most dysregulated pathway in CRC, is associated with cancer cell proliferation, invasion, inflammation, apoptosis, and metastasis (Soleimani et al., 2019). The PI3K/Akt signaling pathway regulates cell survival signals and mediates cell biological progression, and it can affect tumor aggressiveness (Xu et al., 2015). The Rap1 signaling pathway has been shown to be related to endothelial biology and vascular formation (Chrzansowa-Wodnicka, 2013). These results further support the idea that these pathways serve an important role in CRC, which were consistent with the results in our study.

**FIGURE 10** The effects of si-ESM1 and si-SLC10A2 on the proliferation of colon cancer cells. CCK-8 cell proliferation assay results from HCT-116 (A) and SW-480 (B) cells transfected with si-ESM1, si-SLC10A2, or negative control. n = 5 per group, mean values ± SD are presented. P-values were calculated using an Unpaired T-test, *p < 0.05, **p < 0.01.
we constructed a novel 8-gene immune-related signature that can serve as an independent prognostic indicator for CRC. Meanwhile, the prognostic model can be utilized as a predictor for increased immune cell infiltration. This study contributes to our understanding of immune-related genes in CRC and suggests new potential biomarkers for prognosis and therapy. This will help personalize the use of immunotherapies for CRC patients.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

SW and LH finished the major analyses. SW conceived the study and performed the bioinformatics analyses. SW, LH, and ZZ downloaded and organized the clinical and gene expression data. SW, LH, and HM performed the statistical analyses and wrote the manuscript. FL critically revised the article for essential intellectual content. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmolb.2020.604252/full#supplementary-material
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