

## Research

# Analysis of the role of CHPF in colorectal cancer tumorigenesis and immunotherapy based on bioinformatics and experiments

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## Abstract

**Background** Chondroitin polymerizing factor (CHPF) has been found to be involved in the development of numerous cancers and correlated with poor prognosis. However, its role in the tumorigenesis and development of colorectal cancer (CRC) remains unknown.

**Methods** In our research, we explored CHPF expression and clinicopathological characteristics using The Cancer Genome Atlas Program (TCGA), UALCAN, GSE9348, TIMER2.0 and The Human Protein Atlas (HPA) database, in addition, we validated CHPF expression in CRC cell lines by Real-Time Quantitative PCR (qRT-PCR) and Western blot (WB). KM-Plotter, PrognScan and TCGA were also utilized to verify its prognosis value in CRC. Small-interfer RNA (Si-RNA) was used to perform Cell Counting Kit-8 (CCK8), colony formation, 5-ethynyl-2'-deoxyuridine (EDU), transwell and wound healing assays to testify its function on the tumor progression. Based on TCGA database, we probed potential biological mechanism by which CHPF play its role via clusterProfiler package and GEPIA database and we validated their correlation by WB assay. Moreover, we explored its potential association with the tumor microenvironment (TME), immune infiltrated cells, immune checkpoints, tumor mutation burden (TMB) as well as microsatellite instability (MSI), and investigated immunotherapy sensitivity via Tumor Immune Dysfunction and Exclusion (TIDE) algorithm as well as potentially effective therapeutic drugs via pRRophetic algorithm.

**Results** CHPF was identified upregulated in CRC tissues and cells, correlated with poor prognosis, and nodal metastasis status, stage and histological subtype. Down-regulation of CHPF inhibited CRC cell proliferation, migration and its expression correlated with wnt pathway key molecules. In addition, high expression of CHPF was positively correlated with TME scores, Regulatory T cells (Tregs) cell infiltration degree, Programmed death-1 (PD-1), MSI-high (MSI-H), and TIDE scores, however, not with TMB. Targeted drug analysis showed that patients with high CHPF expression were more sensitive to telatinib, recaparib, serdemetan, and trametinib.

Qingyu Song, Pengchao Wang and Jingyu Wu contributed equally to this work.

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**Conclusion** CHPF could promote the proliferation and migration of CRC cells and lead to poor prognosis, possibly through wnt pathways as well as changes in TME. Patients with high expression of CHPF had poor efficacy in immunotherapy, which might be related to Tregs cell infiltration. Above all, it might offer more reliable guidance for future immunotherapy.

**Keywords** Colorectal cancer · CHPF · The tumor microenvironment · Clinical immunotherapy · Tumor progression

## 1 Introduction

Colorectal cancer (CRC) has brought a huge burden to global public health. According to the 2020 global cancer statistics, CRC, which undoubtedly ranks third in the incidence, and second in the mortality [1]. 20% patients of CRC have been in metastatic status at initial diagnosis, and the 5-year survival rate of metastatic CRC patients, with great regret, is less than 20% [2]. Recently, targeted drugs and immunotherapy has brought a ray of hope for metastatic and unresectable CRC [3–6]. However, high rates of recurrence and drug resistance still remain a challenging problem. Hence, it is vital to investigate a novel biomarker to assist diagnosis and clinical treatment.

Chondroitin sulfate (CS), a biomacromolecule, is widespread in the connective tissues of humans, and is extensively applied as a nanocarrier for drug delivery to treat various disease [7–10]. It is verified to be involved in the regulation of massive disease, like osteoarthritis [11], cardiovascular disease [12], malignant disease [13], and etc. Furthermore, CS could interact with various molecules, such as cytokines, chemokines and growth factors, in addition, it could also exert antitumor effects through regulating immune cells infiltrated and angiogenic inhibition. The biosynthesis of CS requires massive glycosyltransferases to participate in. Chondroitin polymerizing factor (CHPF) is a crucial molecule of them to promote the extension of CS, consisted of 775 amino acids, and acts as a type II transmembrane protein. CHPF could not only contribute to the biosynthesis of CS, it has also been demonstrated that it could affect the development of several human cancers. For example, Lin et al. elucidated that CHPF could increase expression and stability of E2F1 protein through UBE2T-mediated ubiquitination to promote the tumorigenesis of gastric cancer [14]. In malignant melanoma, Sun et al. discovered that CHPF could promote cancer development via upregulating the cell cycle protein “CDK1” [15]. Instead, studies in hepatocellular carcinoma seemed to be entirely contradictory [16, 17]. However, to our knowledge, what exactly is the role that CHPF plays in CRC has yet never been reported.

Accumulating evidence indicates the development of cancer and clinical immunotherapy efficacy were closely associated with the tumor microenvironment (TME) [18, 19]. Meanwhile, tumor mutational burden (TMB), immune checkpoints, and microsatellite instability (MSI) have been proved as essential factors to directly influence immune cells infiltrated and immunotherapy outcomes [20–22]. Thus, a thorough exploration of above factors is vital for better clinical management of patients with CRC.

In present paper, by the combination of bioinformatics and basic experiments approach, we verified CHPF expression and survival prediction value in CRC, explored its tumorigenic role, investigated the mechanism by which it might be involved in to regulate the CRC progression, probed potential association between CHPF mRNA and TME, Tumor infiltrating immune cells (TIICs), immune checkpoints, TMB, MSI, and clinical therapy. Especially importantly, as far as we know, our study was the first to investigate the role of CHPF in CRC through bioinformatics and experiments.

## 2 Materials and methods

### 2.1 Data acquisition

We obtained TCGA–CRC cohort data (containing RNA-sequencing and clinicopathological characteristics of colon adenocarcinoma and rectal adenocarcinoma) from The Cancer Genome Atlas Program (TCGA) database (<https://portal.gdc.cancer.gov/>) for subsequent series of analyses (FPKM format) (which contains 488 CRC tissues and 42 normal colorectal tissues data). Furthermore, GSE9348 dataset obtained from GEO database (<https://www.ncbi.nlm.nih.gov/gds/>) was utilized to acquire CRC gene expression data (which contains 70 tumor tissues of CRC patients and 12 normal colorectal tissues).

## 2.2 Analysis of CHPF expression

The differential expression of CHPF mRNA between pan-cancers and their matched normal tissues were analyzed with TIMER2.0 (<http://timer.cistrome.org/>) database in our study [23]. This database is mainly dedicated to the analysis of tumor immune infiltration and differential gene expression. We also analyzed CHPF mRNA expression in TCGA cohort and GSE9348 via R program. Meanwhile, we identified CHPF protein expression levels via UALCAN database (which covering CTPAC data) (<https://ualcan.path.uab.edu/index.html>) and The Human Protein Atlas (HPA) database (which containing immunohistochemistry images) (<https://www.proteinatlas.org/>) [24, 25].

## 2.3 Clinicopathological correlation and survival analysis

UALCAN database were utilized to explore the correlation between CHPF mRNA expression and various clinicopathological features of TCGA database, such as stage, histological subtype, nodal metastasis status, etc. Moreover, the survival value of CHPF expression in CRC patients were analyzed by PrognoScan (GSE17536) (<http://dna00.bio.kyutech.ac.jp/PrognoScan/>) [26], Kaplan–Meier Plotter (<http://kmplot.com/analysis/>) [27], as well as TCGA CRC cohort.

## 2.4 Functional enrichment analysis

Differential expression genes (DEGs) were identified according to the median value of CHPF expression of 488 CRC patients by the limma package ( $|\text{LogFC}| > 1, P < 0.05$ ). Then “clusterprofiler” package was applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis ( $P < 0.05$ ). Among them, GO analysis contains biological process (BP), cellular component (CC), molecular function (MF). Meanwhile, GEPIA database (<http://gepia.cancer-pku.cn/index.html>) was applied to perform correlation analysis.

## 2.5 Analysis of TME

Based on the median value of CHPF expression in TCGA–CRC tissues, we divided 488 patients into high- and low-expression subsets. The Estimate algorithm was used to compare the differential level of stromal cells, immune cells, as well as the tumor purity between the two subsets. We further calculated the proportions of 22 TIICs in each CRC sample and next conducted differential expression analysis of 22 TIICs between the two subsets and performed correlation analysis between them and CHPF expression. Moreover, we compared the immune checkpoints, MSI status between the two subsets, and performed spearman correlation analysis between the TMB and CHPF expression.

## 2.6 Immunotherapy sensitivity and targeted drugs screening

The Tumor Immune Dysfunction and Exclusion (TIDE) algorithm (a model which established based on the combination of T cell dysfunction and exclusion feature) was utilized to predict the potential immunotherapy response of the two subsets of CRC patients. Meanwhile, pRRophetic algorithm was applied for screening potential clinical therapeutic targeted drugs sensitive to different subsets.

## 2.7 Cell culture and small interfer-RNA (Si-RNA) transfection

CRC cell lines (SW480, SW620, HCT-116, DLD1) and normal intestinal epithelial cells (NCM460) were purchased from Shanghai cell bank (Shanghai, China) and cultured in RPMI 1640 (BI, Israel) containing 10% fetal bovine serum (FBS, BI, Israel). Three CHPF Si-RNA and negative NC were purchased from GenePharma (Shanghai, China). SiRNA transfection was conducted by Lipofectamine 3000 reagent according to the manufacturer's instructions (Thermo Fisher Scientific, USA) when the cells adhesion reached 70%. 6 h later, the cells were washed via using PBS and incubated for 24 h. And after that, the transfected cells could be digested and used for subsequent cell experiments.

## 2.8 Real-time quantitative PCR (qRT-PCR)

Total RNA was extracted via Trizol method, the RNA concentration was detected by using NanoDrop 2000, and the RNA was reverse transcribed into cDNA with HiScript III RT SuperMix for qPCR (Vazyme, China). SYBR Green Master Mix (Vazyme, China)

**Fig. 1** CHPF is upregulated in pan-cancers and CRC. **A** The expression of CHPF in pan-cancers based on TIMER2.0. **B–D** The expression of CHPF in CRC according to TCGA and GSE9348. **E** Protein level of CHPF in CPTAC. **F, G** Immunohistochemistry images of colon and CRC tissues obtained from HPA database. **H** CHPF mRNA expression level in CRC cell lines. **I** WB depicted CHPF protein level in CRC cell lines (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

was used to measure the CT value of the gene in the cell. The CHPF expression was calculated and normalized to  $\beta$ -actin using the  $2^{-\Delta\Delta CT}$  method. The primers used for qRT-PCR were listed in Supplementary Table S1.

## 2.9 Western blot (WB)

The protein of CRC cells was extracted via using RIPA lysis buffer (Beyotime, China). The protein concentration was detected by using BCA Protein Assay Kit (Beyotime, China). Next, the proteins were separated by SDS-PAGE, and further transferred to PVDF membrane. Then the membranes were incubated with 5% skimmed milk for 2 h at room temperature and washed with TBST. The membranes were incubated with diluted primary antibodies overnight at 4 °C and then washed. Next the membranes were blocked with secondary antibodies for 1 h. All antibodies used in our study are listed in Supplemental Table S2.

## 2.10 Cell counting Kit-8 (CCK8) assay

The transfected cells were seeded into 96-well plate ( $3 \times 10^3$  cells/well) with antibiotic-free medium and incubated for 0, 24, 48, 72 h. at each point, we added 10  $\mu$ l CCK-8 reagent (Vazyme, China) into each well and incubated at 37 °C for 2 h. Then we detected the absorbance of each well at 450 nm by a microplate reader.

## 2.11 5-Ethynyl-2'-deoxyuridine (EdU) assay

The transfected cells were seeded into 6-well plates ( $1 \times 10^4$  cells/well) and cultured to normal growth stage. Then EDU reagent (RiboBio, China) was added into each well and incubated for 2 h. Then the cells were fixed with 4% paraformaldehyde, penetrated with 0.5% TritonX-100, and stained with Apollo Dye Solution and Hoechst 33342 solution for 30 min. Then the images were photographed and the EDU-positive cell rate (%) was calculated.

## 2.12 Colony formation assays

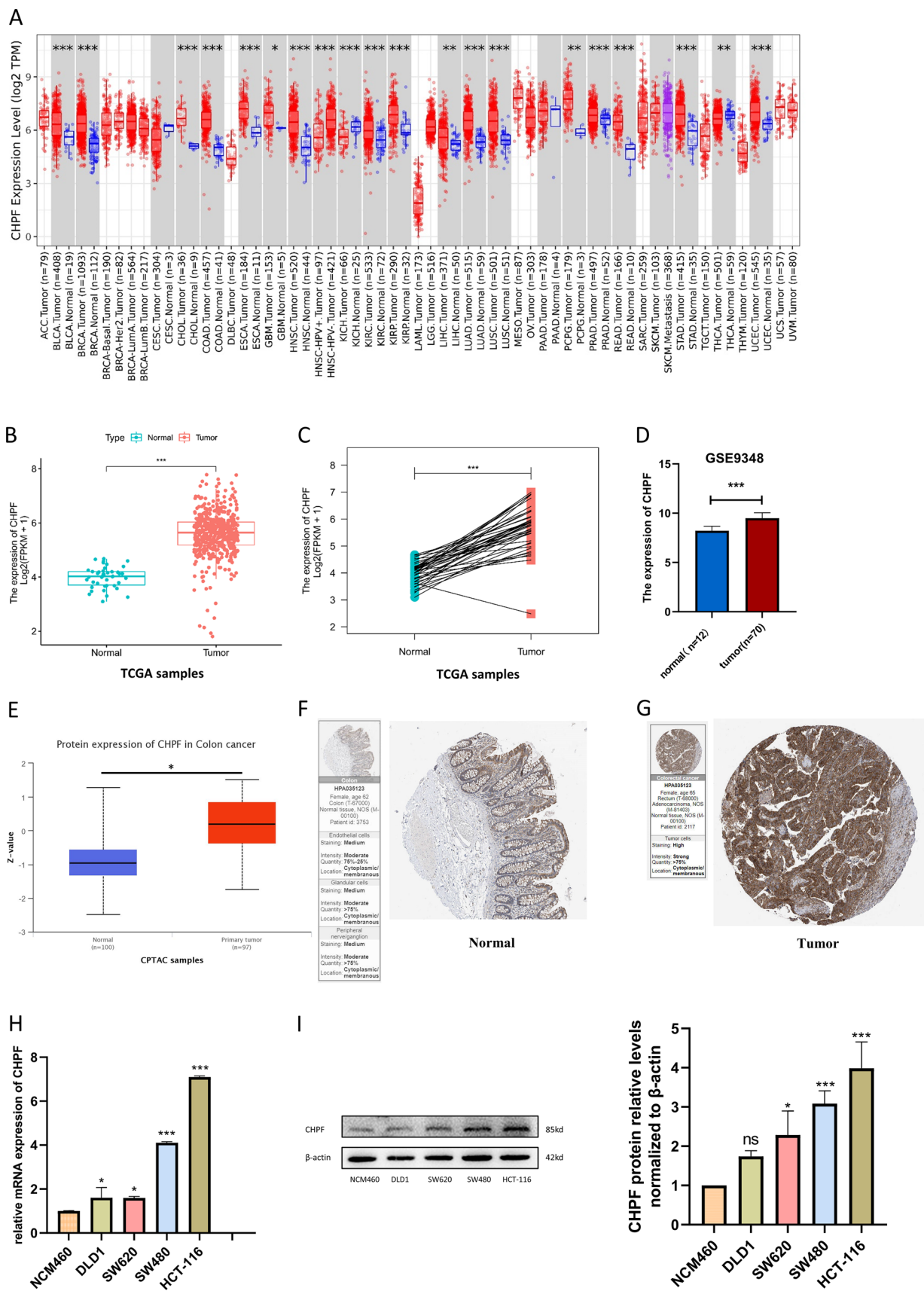
The transfected cells were seeded into 6-well plates (800 cells/well) and incubated at 37 °C for 2 weeks. Then we washed the plates, fixed and visualized the cells with 0.1% crystal violet staining for 20 min. The images were then photographed and the number of colonies was counted.

## 2.13 Wound-healing assay

The transfected cells were seeded into 6-well plates and incubated until the cell adhesion reached 80%, a 10  $\mu$ l tip was applied to create a wound and the cells were washed with PBS. Then the cells were incubated with serum-free medium for 48 h, observed and photographed at 0 h and 48 h.

## 2.14 Transwell assay

The transfected cells were seeded into the upper chambers of the transwell plates ( $5 \times 10$  cells/well) (Coring, USA) with serum-free medium. The bottom chambers were added with 600  $\mu$ l complete medium. After 48 h, the cells were fixed and visualized by 0.1% crystal violet staining for 20 min at room temperature.



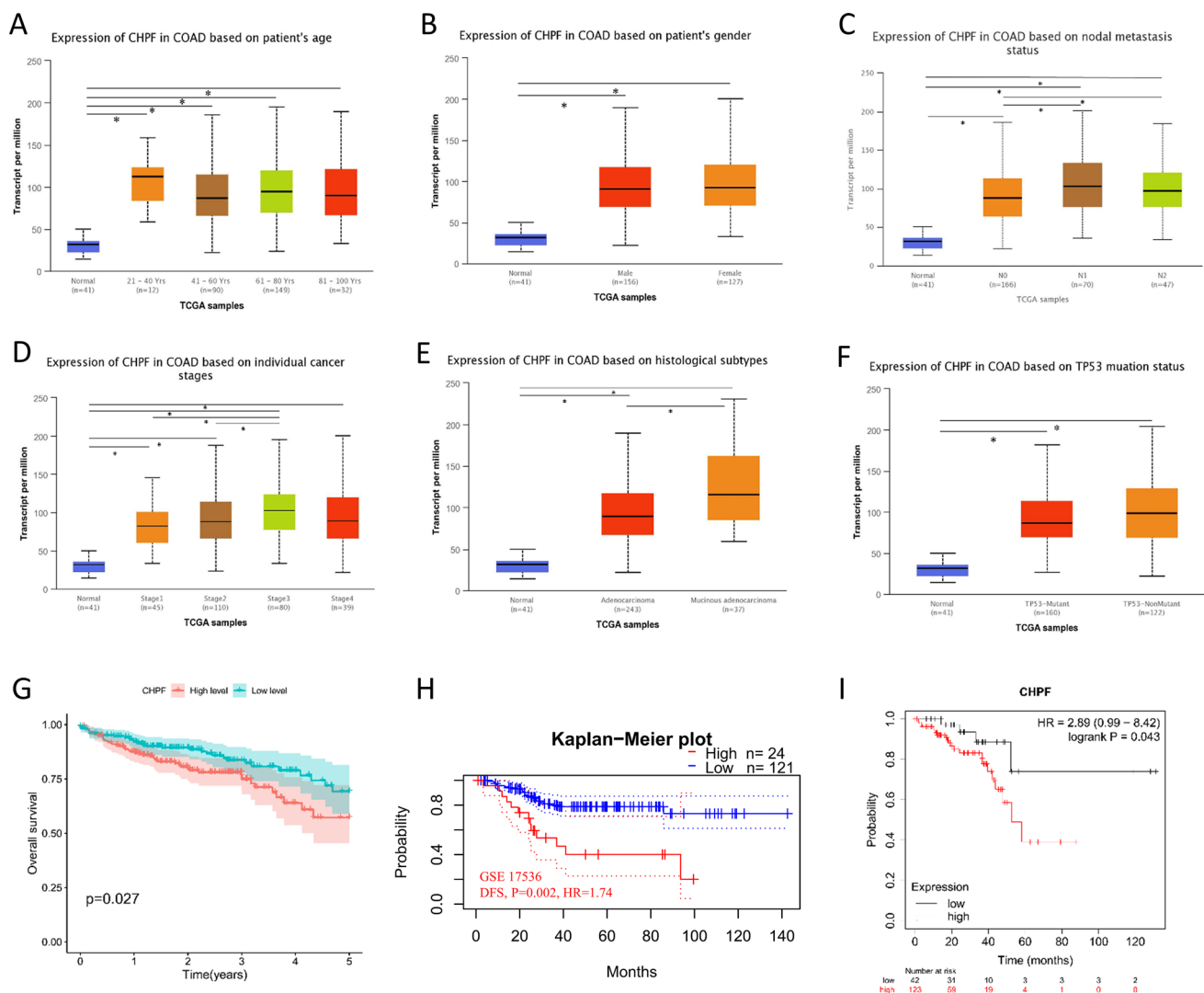
### 3 Results

#### 3.1 CHPF expressed higher in CRC tissues and cell lines

To determine the differential expression of CHPF in tumor and their matched normal tissues comprehensively, we first performed a pan-cancer analysis using TIMER 2.0 database. As depicted in Fig. 1A, CHPF expressed higher in 15 cancers (including CRC), when compared with normal tissues. Since it has not been studied in CRC, hence, we chose it for next studies. TCGA data, GSE9348, CPTAC and HPA database revealed CHPF expression notably upregulated in CRC tissues (Fig. 1B–G). Subsequently, we performed qRT-PCR and WB in CRC cell lines, all experiments outcomes revealed that CHPF actually expressed higher in CRC both in mRNA and protein level (Fig. 1H, I).

#### 3.2 Clinicopathological features and prognostic value of CHPF

We applied UALCAN database to investigate the correlation between CHPF expression and clinicopathological parameters of COAD. As depicted in Fig. 2A–F, CHPF mRNA expression seemed to definitely correlated with nodal metastasis



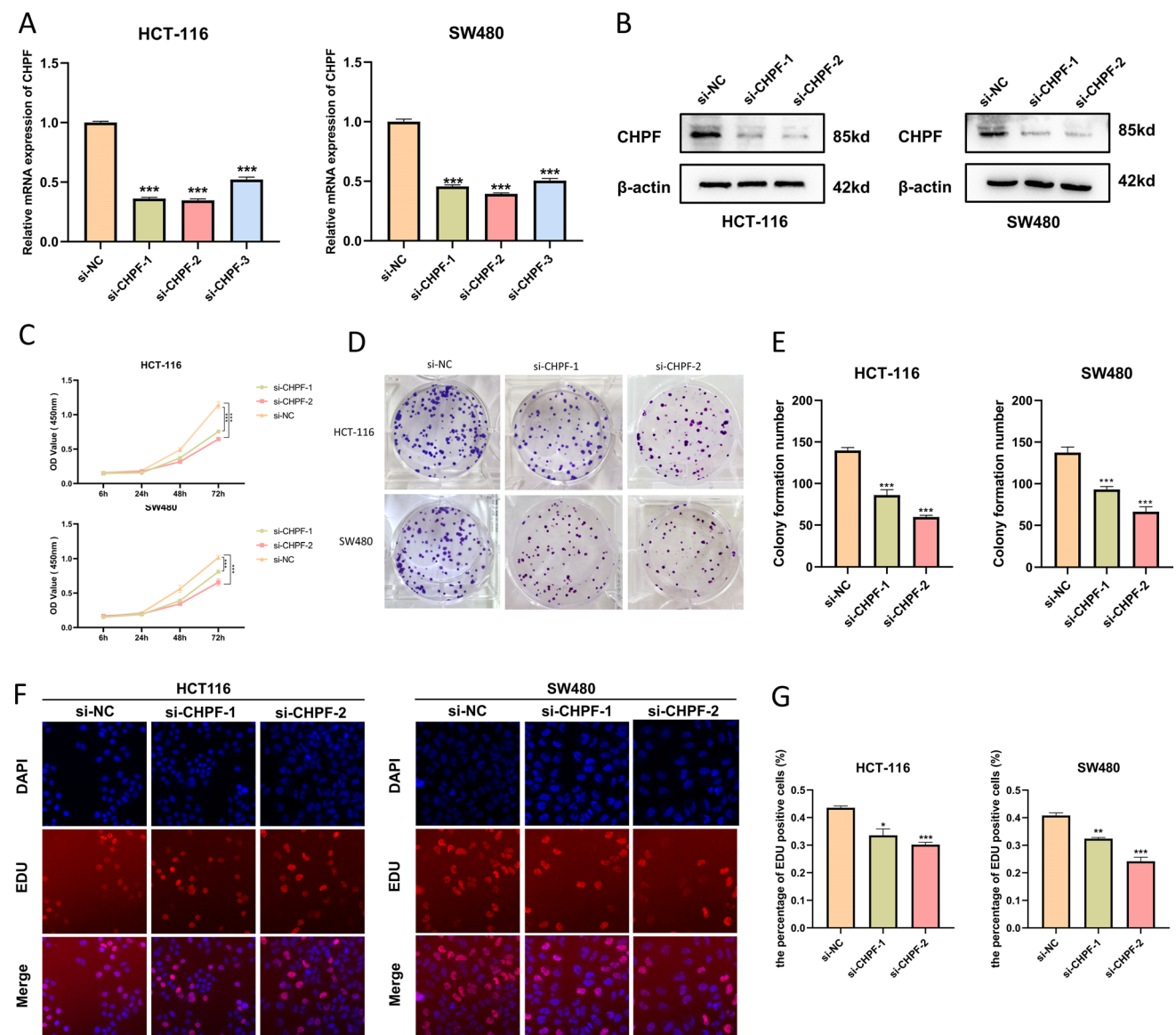
**Fig. 2** Correlations between CHPF and clinicopathological features and prognosis. **A–F** CHPF expression level in different clinical characteristics stratification. **G** Prognosis value of CHPF in TCGA. **H** Prognosis value of CHPF in GSE17536. **I** Prognosis value of CHPF in Kaplan–Meier Plotter (\* $P < 0.05$ )



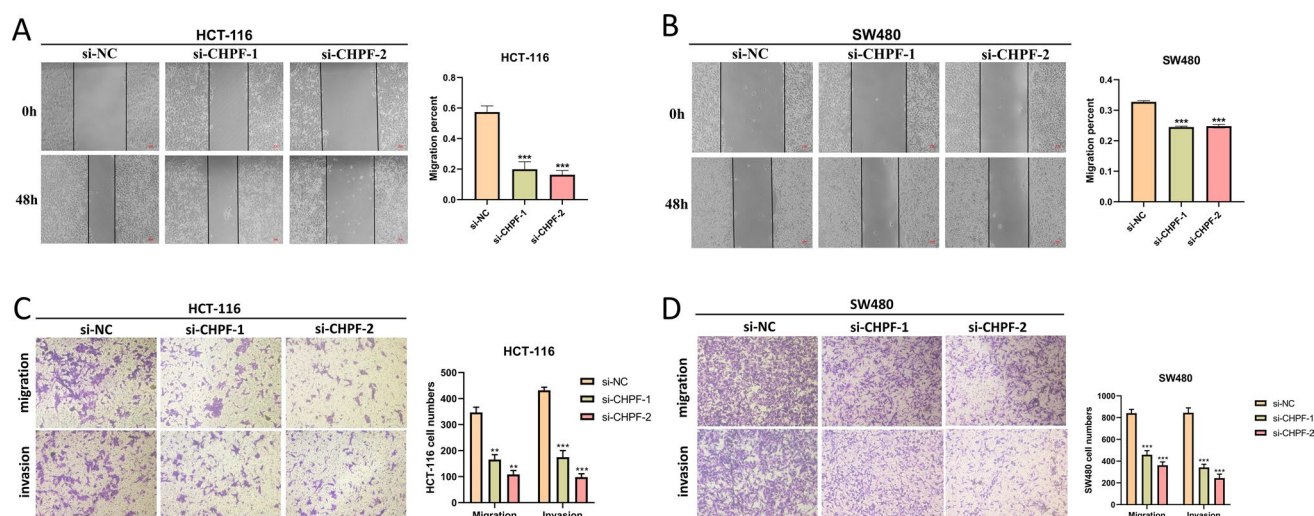
status, stage and histological subtypes. However, there was no significantly difference between CHPF expression and age, gender as well as TP53 mutation status. To verify the prognosis value of CHPF, we searched three platforms (TCGA, GSE17536 and KM-Plotter), all results displayed high level of CHPF might be in correlation with poor survival (Fig. 2G–I). Hence, we could infer that CHPF might be a potential indicator to predict the prognosis of CRC.

### 3.3 CHPF promotes CRC cell proliferation and metastasis in vitro

Based on the results of CHPF expression in CRC cell lines, we selected HCT-116 and SW480 for following functional experiments. Knockdown efficiency of siRNA for CHPF was testified by qRT-PCR and WB (Fig. 3A, B). The CCK8, colony formation and EDU assays demonstrated the cell proliferation rate was remarkably inhibited when HCT-116 and SW480 transfected with si-CHPF (Fig. 3C–G). Wound healing and transwell assays indicated the downregulation of CHPF expression could remarkably suppressed the migration and invasion ability of CRC cells (Fig. 4A–D).



**Fig. 3** CHPF promotes the proliferation ability of HCT-116 and SW480 cells. **A, B** The knockdown efficiency identified by qRT-PCR and WB. **C** The effect of CHPF on proliferation exhibited by CCK8 assay. **D, E** The effect of CHPF on proliferation exhibited by colony formation assay. **F, G** The effect of CHPF on proliferation exhibited by EDU assay (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )



**Fig. 4** CHPF facilitates migration and invasion ability of CRC cells. **A, B** The effect of CHPF on migration detected by wound healing assay. **C, D** The effect of CHPF on migration and invasion detected by transwell assay (\*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

### 3.4 CHPF knockdown inhibits wnt-pathway in CRC

To explore the potential function of CHPF, we performed GO and KEGG enrichment analysis on DEGs which were screened between high- and low-expression subsets of TCGA. Firstly, GO annotation indicated DEGs we mainly enriched in processes such as “complement activation”, “phagocytosis, engulfment”, “immunoglobulin complex”, etc. (Fig. 5A). Meanwhile, KEGG pathway enrichment analysis revealed “estrogen”, “wnt signaling pathway”, “gastric cancer”, “signaling pathways regulating pluripotency of stem cells”, etc., were significantly enriched (Fig. 5B). Moreover, GEPIA data indicated a positive correlation between 2 key genes of wnt-pathway ( $\beta$ -catenin and c-Myc) and CHPF expression (Fig. 5C). Consistent with the above, WB results showed the expression of  $\beta$ -catenin and c-Myc significantly downregulated when we knockdown CHPF expression (Fig. 5D).

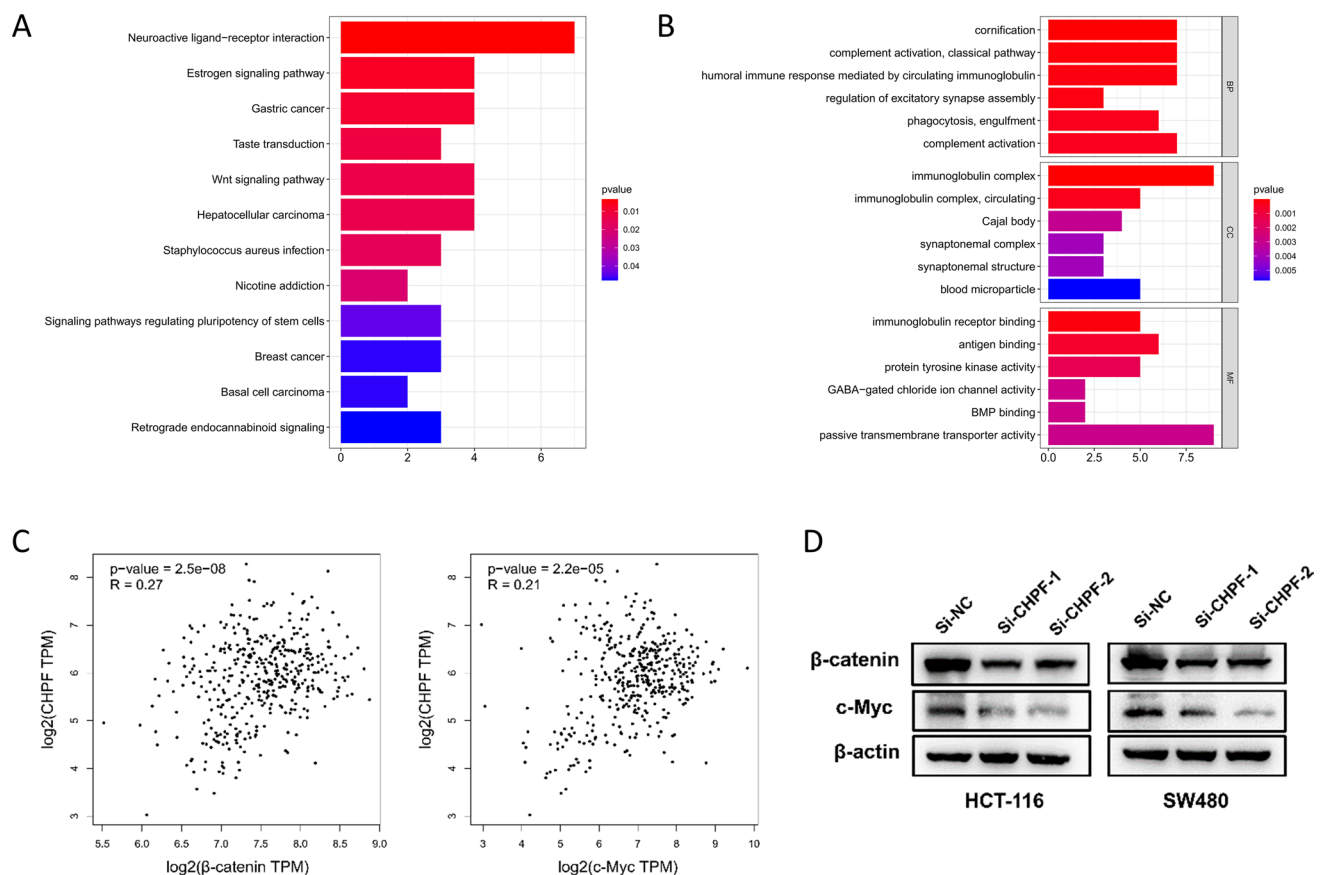
### 3.5 CHPF might be positively correlated with TME and Tregs infiltration

Based on the results of enrichment analysis, we considered CHPF expression might influence TIICs and might be involved in the process of reshaping the TME. So, we explored the relationship between CHPF and TIICs to understand its role in TME and facilitate future hopeful immunotherapy development. We found the CHPF high-expression subset showed the higher infiltration level of stromal and immune cells, and it also exhibited more heterogenous cell populations, which might represent the high-expression subset was in a higher immune infiltration status (Fig. 6A). Moreover, 3 TIICs such as T cells CD4 resting, dendritic cells resting, dendritic cell activated were identified to have a lower expression in the CHPF high-expression subset, while T cells regulatory (Tregs) and neutrophils exhibited the opposite trends (Fig. 6B). Spearman's correlation analysis between 22TIICs and CHPF revealed Tregs was most positively correlated with CHPF expression (Fig. 6C, D). Tregs has been verified vital in hindering antitumor immune responses. Specific depletion as well as functional alteration of Tregs could evoke effective tumor immunity [28]. Hence, CHPF might induce Tregs cells infiltration in CRC to promote the tumor development.

### 3.6 CHPF might be positively correlated with PD-1 and MSI-H

According to previous studies, the proportion and functional status of infiltrated immune cells, the expression of immune checkpoints, TMB level, as well as MSI-high (MSI-H) status could be correlated with the efficacy of immune checkpoint inhibitors. In our study, the differential expression of immune checkpoints showed that TNFRSF25, CD70, TNFRSF8, TNFRSF4, TMIGD2, CD276, CD27, most importantly, Programmed death-1 (PD-1), and etc. were significantly





**Fig. 5** CHPF knockdown inhibit wnt-pathway in CRC. **A, B** KEGG and GO analysis of CHPF based on TCGA cohort. **C** The correlation between CHPF expression and  $\beta$ -catenin and c-Myc according to GEPIA. **D** Protein level of  $\beta$ -catenin and c-Myc detected by WB when we knockdown CHPF expression (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

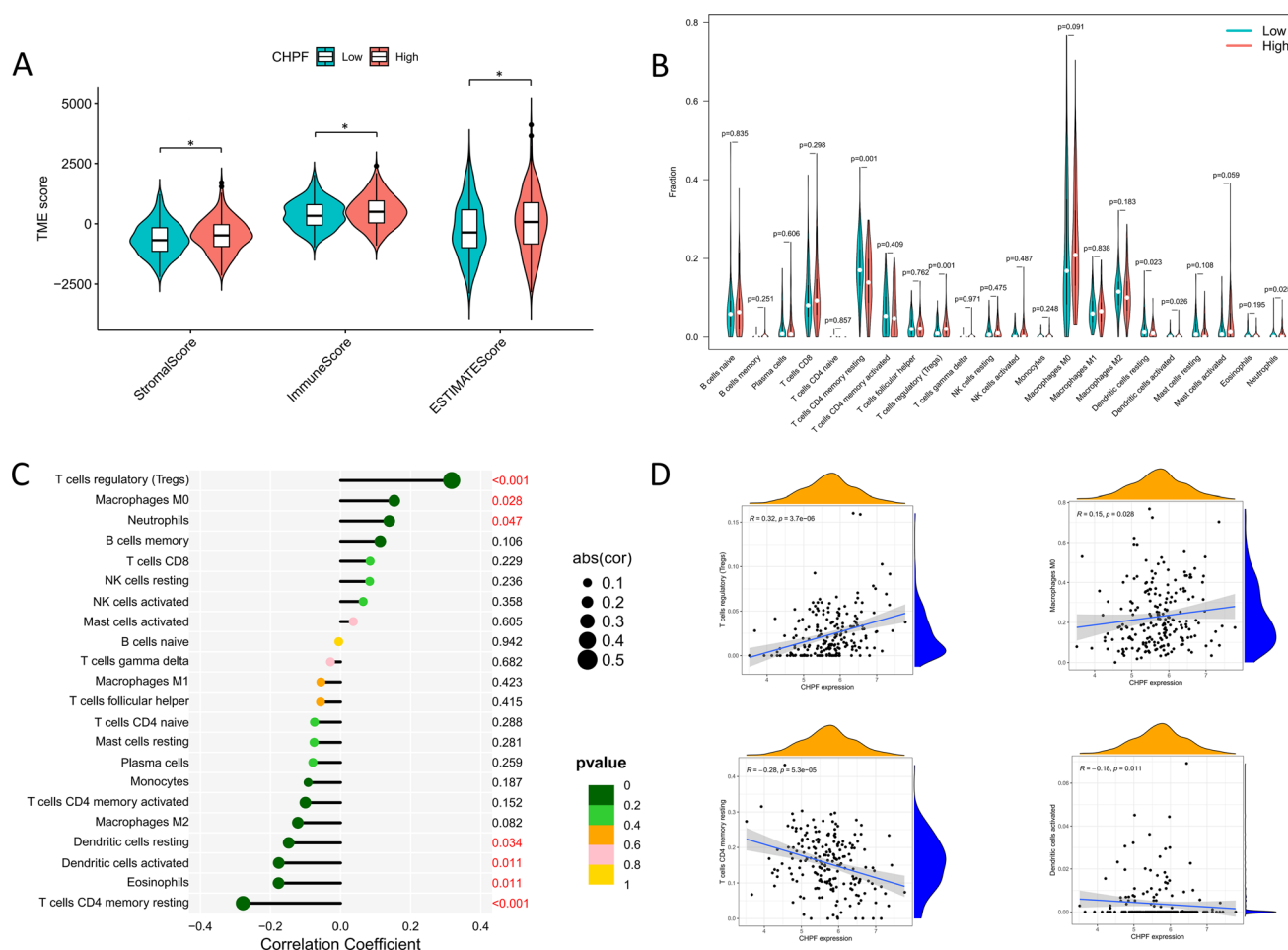
upregulated in the CHPF high-expression subset (Fig. 7A). Moreover, the scatter diagram revealed there was no significant correlation between TMB and CHPF expression (Fig. 7B). In addition, it seemed to have a relatively higher percentage of MSI-H in the high-expression subset (Fig. 7C, D).

### 3.7 Immunotherapy efficacy prediction and targeted drugs screening

To investigate the response of CRC patients with different CHPF expression to immunotherapy, we performed analysis on the two subsets with TIDE algorithm, and we could find the low-expression subset might be likely to benefit more from immune checkpoint inhibitors (Fig. 8A). Furthermore, we attempt to explore potential targeted drugs applicable to CRC patients with different CHPF expression based on pRRophetic algorithm. As a result, we identified 4 drugs (telatinib, recaparib, serdemetan, and trametinib) sensitive to high-expression subset (Fig. 8B). Meanwhile, there were 25 drugs we screened more sensitive to the low-expression subset (Supplemental Figure S1). All in all, above analysis provided more possible strategies to improve the treatment efficacy of CRC.

## 4 Discussion

Transcriptomic gene expression analysis and various bioinformatic analysis tools provides us substantial assistances in searching biomarkers, comprehensively understanding the function of these molecules, and exploring the interactions between molecules as much as possible [29–31]. CHPF has been reported to participate in the development of several cancers. For example, in gastric cancer, CHPF could regulate E2F1 expression to influence its proliferation and migration [14]. Moreover, it was also validated to play essential roles on the development of breast cancer, lung cancer, malignant

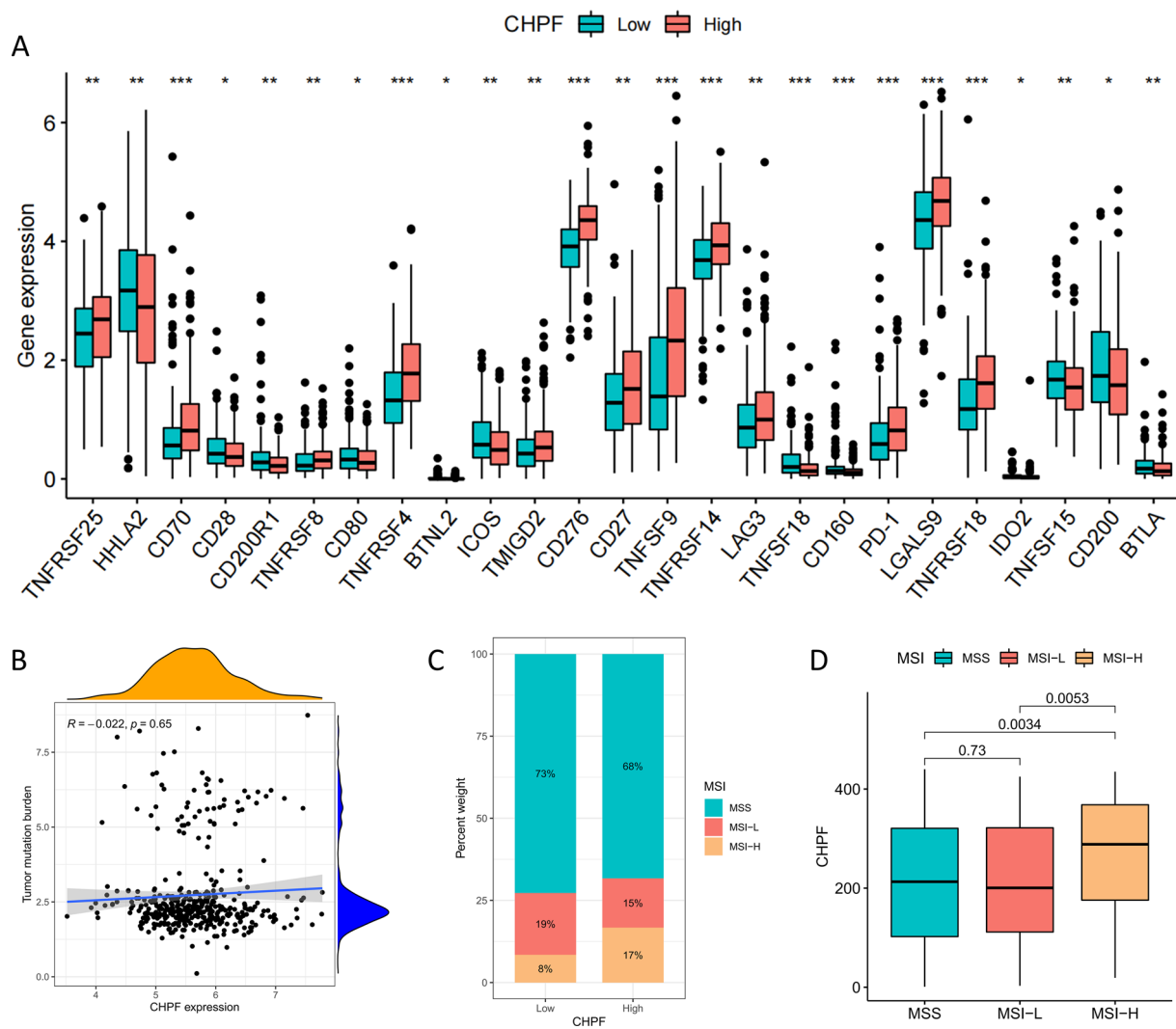


**Fig. 6** Correlations analysis of TME and TIICs. **A** The score of TME between high- and low-expression subsets. **B** Abundance difference of 22 TIICs between the two CRC subsets. **C, D** Correlations between CHPF gene expression and the abundance of immune cells (\* $P < 0.05$ )

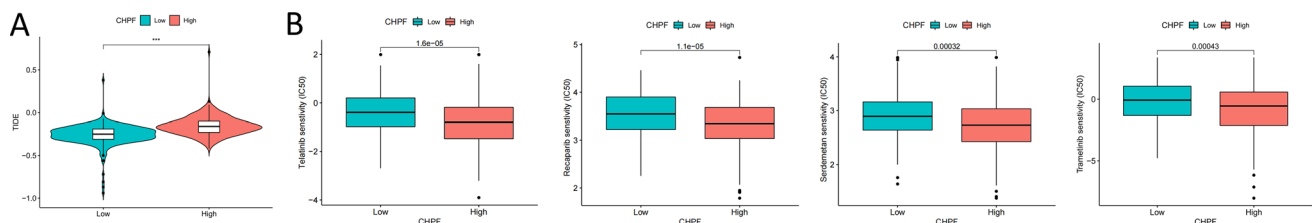
melanoma, hepatocellular and etc. Meanwhile, Wu et al. demonstrated CHPF was significantly upregulated in CRC, but they didn't explore which role the gene CHPF might play in CRC [32]. In our study, we analyzed CHPF expression and prognostic value in CRC based on bioinformatics and basic experiments. Meanwhile, we investigated the relationship between CHPF expression and TME, TMB, MSI, immune checkpoint inhibitors (ICIs) response and possible sensitive targeted drugs. Notably, we conducted numerous experiments to identify the oncogenic roles CHPF play in CRC cell lines.

In our study, CHPF was demonstrated upregulated in CRC tissues and cell lines. Meanwhile, with the help of TCGA dataset, Kaplan–Meier Plotter and GSE17536, CHPF gene was identified significantly linked with CRC poor prognosis. Moreover, clinicopathological correlation analysis indicated CHPF was independent of age, sex, and TP53 mutation status, but was markedly correlated with nodal metastasis status, stages, and histological subtypes. Based on this, we conjectured that the higher expression of CHPF, the more likely the tumor is to develop lymph node metastasis. We performed functional experiments in HCT-116 and SW480, the results revealed knockdown of CHPF could suppress cell proliferation, migration and invasion. Enrichment analysis and gepia data showed wnt-pathway was significantly associated with CHPF. Consistently, WB assay displayed  $\beta$ -catenin and c-Myc was down-regulated after we knockdown CHPF expression. Therefore, we could simply infer that CHPF exerts its oncogenic effects through wnt-pathway. Nevertheless, the specific mechanisms involved require further elucidation.

Interactions between cancer cells and TME could influence cancer development, migration, clinical therapy and drug resistance [33, 34]. In general, TME mainly consists of various immune cells, extracellular matrix (ECM) and secreted factors. Among them, Tregs cells have been extensively demonstrated to suppress self-antigen response, thus acting as a barrier to anti-tumor immune response [28, 35]. In addition, there has been extensive research surrounding the regulation of the TME to improve tumor progression [36]. In our study, we estimated the correlation between immune scores, stromal scores, and the content of 22 immune cells and CHPF expression. Thus, we found Tregs cells expression were significantly



**Fig. 7** Correlation analysis of immune checkpoints, TMB and MSI. **A** Expression of immune checkpoints between high- and low-expression subsets. **B** Correlations between CHF gene expression and TMB. **C** Percentage of different MSI status in the two subsets. **D** The expression of CHF in different MSI status groups



**Fig. 8** Immunotherapy efficacy prediction and targeted drugs screening. **A** TIDE scores between the two subsets. **B** Potentially effective targeted drugs between the two subsets (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )

upregulated in the CHF high-expression subset and was positively correlated with CHF expression, which seems to partially explain why CHF high-expression populations have relatively poor prognosis.

Recently, ICIs have been extensively applied in clinical management and have improved survival outcomes in many malignancies [37, 38]. Meanwhile, tumor with higher level of TMB, MSI-H, neo-antigen load, as well as immune checkpoints have been considered to stimulate more T-cell recognition, and strongly associated with better clinical prognosis [39–41]. In our study, we performed a series of analysis to explore the potential relations between CHF and existing biomarkers of ICIs which have been widely proven, such as TMB, MSI-H and immune checkpoints in CRC. For example,

we attempted to explore the correlation of CHPF with 47 immune checkpoint-related genes and the results showed that a total of 25 genes were differentially expressed, 13 of which were upregulated in the CHPF high-expression subset (including PD-1). It is noteworthy that we didn't observe the most discussed and clinically applied molecules, such as PD-L1 (CD274), PD-L2 (PDCD1LG2) and CTLA4 in these 25 genes. Moreover, CHPF has been demonstrated expressed differentially between MSI-H and MSS, MSI-L. Importantly, ICIs have been improved by FDA for the treatment of MSI-H CRC patients. Meanwhile, MSI-H is the only one of the above factors currently applied in clinical practice to determine whether a patient suitable for ICB treatment, which further emphasizes its importance. However, we found that TMB didn't seem to distinctly correlate with CHPF expression. Interestingly, the TIDE results indicated that patients with higher CHPF expression appear to be more prone to immune escape and less effective in ICIs. In other words, patients with CHPF low-expression are more likely to benefit from ICIs. Thought-provokingly, according to the results of the MSI and PD-1 expression analysis, it would be logical that CHPF high-expression subset should be more likely to benefit from ICIs, yet the TIDE analysis yielded the opposite result. The previous study perfectly explained this seeming contradictory phenomena. Despite the higher level of biomarkers in CHPF high-expression subset, which induced higher tumor antigen recognized by antigen presenting cells, then there are fewer CD8<sup>+</sup>T cells and more Tregs cells infiltrated in TME. Due to no effector cells play roles in killing tumor cells, thus inhibiting the body's response to ICIs. Jiang et al. believed that in addition to the four factors mentioned above, there were many factors could influence the outcome of immunotherapy, such as antigen presentation defects, interferon signaling, intestinal microbiota, as well as tumor aneuploidy etc [42]. Meanwhile, TIDE algorithm was established mainly on the basis of integration of the expression signature of T cell dysfunction and T cell exclusion. Therefore, we assumed that the results obtained by the TIDE algorithm could be applied as a tool to predict the reflection of ICIs in clinical practice, but not absolutely, we need to consider many other factors equally importantly. Last but not least, we have obtained some targeted drugs based on pRRophetic algorithm for different expression of CHPF, in order to hopefully better guide the clinical dosing decisions.

In summary, we conducted comprehensive analysis to investigate the expression patterns, prognosis value, correlations between TME, immunotherapy evaluation and targeted drugs prediction, what's more, experiments were performed to confirm the CHPF expression, its effect on CRC cells proliferation and migration and it might be associated with wnt/ $\beta$ -catenin pathway. All above analysis and experiments might offer a novel insight into the relationship between CHPF and CRC, uncovering new therapeutic targets as well as predictive biomarkers for CRC. Furthermore, our study was the first comprehensive bioinformatics analysis of CHPF in CRC and experimental validation of its differential expression in CRC, as well as exploring its vital role in the proliferation, migration and invasion of CRC.

There are still some weaknesses in our study, for example: [1] lack of animal experimental validation [2]. How gene CHPF plays a role in promoting proliferation and migration of CRC cells through wnt/ $\beta$ -catenin pathway [3]. On closer consideration, we initially explored the relationship between CHPF and TME only through a bioinformatics approach, lacking relevant experimental evidence to verify the associations between them. The above deficiencies offer us more directions for future research to explore and require more efforts.

## 5 Conclusion

In conclusion, our study demonstrated CHPF significantly upregulated in CRC and associated with poor prognosis, could contribute to cell proliferation and metastasis via wnt-pathway and Tregs infiltration to regulate the malignancy of CRC cells. Furthermore, patients with high expression of CHPF might benefit less from ICIs. Above all, our study suggested that CHPF might be a promising biomarker for influencing the prognosis of CRC patients, providing a novel perspective for CRC immunotherapy.

**Author contributions** Q.S, P.W and J.W contributed equally to this study. Q.S, P.W and J.W conceived and designed the study, curated and analyzed the data. M.L, Q.X, Y.S, Z.W, X.M, and Q.Z reviewed and edited the manuscript. All authors had final approval of the submitted versions.

**Data availability** All data in our study could be obtained from TCGA, and GEO database.

## Declarations

**Competing interests** The authors declare no competing interests.

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