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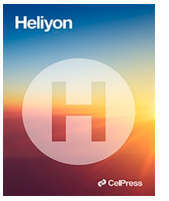

IL-8-mediated overexpression of ZNF274 promotes the proliferation and migration of colorectal cancer cells through the transactivation of MRPL40

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ABSTRACT

Background: Colorectal cancer (CRC) is one of the most prevalent malignant tumors with high morbidity and mortality rates worldwide. ZNF274, a member of the zinc-finger-protein family of transcription factors, is critical in chromosomal remodelling and tumorigenesis. However, the role of ZNF274 in CRC and the underlying molecular mechanisms remain unclear.

Methods: Immunohistochemical analysis was performed to quantify the expression of ZNF274 in human CRC tissues. The Kaplan–Meier method was used to analyse the relationship between ZNF274 expression and CRC prognosis. The correlation between ZNF274 expression and clinical features was analyzed using Cox regression analysis. Cell proliferation and migration were evaluated by CCK-8, colony formation, and Transwell assays. The limma R package was used to analyse IL-8-related differentially expressed genes in the GSE30364 dataset. The DAVID method was used to screen significantly enriched pathways. Chromatin immunoprecipitation (ChIP)-qPCR and luciferase reporter assays were performed to determine the transcriptional regulation of MRPL40 by ZNF274.

Results: ZNF274 was overexpressed in CRC tissues and indicated poor prognosis. High ZNF274 expression was linked to larger tumor size, invasion, lymph node metastasis, and AJCC stage. Ectopic expression promoted CRC cell proliferation and migration. Mechanistically, MRPL40 was identified as the direct target gene that transactivates the expression of ZNF274. Moreover, IL-8 upregulated ZNF274 expression in a dose-dependent manner. Downregulation of either ZNF274 or MRPL40 expression abrogated the effect of IL-8 on promoting the proliferation and migration of CRC.

Conclusion: This study revealed an oncogenic role of ZNF274 and the mechanism by which ZNF274 participated in IL-8-induced promotion of CRC progression. These findings demonstrate that ZNF274 could be used as a prognostic factor and potential therapeutic target for CRC treatment.

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1. Introduction

Colorectal cancer (CRC) is one of the most fatal malignancies worldwide. Developing new therapeutic targets for CRC treatment is a major research focus [1,2]. Interleukin-8 (IL-8) (also known as chemokine CXCL8) plays a crucial role in the inflammatory response and is currently a target for cancer treatment [3]. Overexpression of IL-8 is associated with higher stage, grade, and burden of various cancer types. Recent studies have revealed the important function of IL-8 throughout the whole process of CRC development and progression [4,5]. IL-8 expression is activated in the cancer stem cell niche from the precancerous adenoma stage of CRC and can predict the recurrence of stage III CRC [4,6,7]. IL-8 is also involved in glycyrrhizin-promoted liver metastasis of CRC [8]. However, the mechanism by which IL-8 promotes the progression of CRC needs further investigation. In our study, we found that IL-8 stimulation markedly increased mitochondrial gene expression, implying that mitochondrial bioavailability could be a critical factor for IL-8-mediated CRC progression.

Zinc finger proteins (ZNFs) are the most abundant transcription factors essential in the proliferation, differentiation, and apoptosis of cells [9,10]. Aberrant expression of ZNFs impacts the invasion, migration, and metastasis of cancer [10]. As one of the most prominent ZNF proteins, ZNF274 possesses five zinc finger C2H2 domains, two Kruppel-associated box domains, and a leucine-rich KRAB-a domain [11]. ZNF274 can directly bind with target gene promoters via the zinc finger domain [11]. In addition, it recruits chromatin-modifying proteins, such as SETDB1 and TRIM28/KAP1, leading to transcriptional repression of SNORD116 [12]. ZNF274 and SETDB1 H3K9 methyltransferases inhibit SNORD116 expression in Prader-Willi syndrome (PWS)-related induced pluripotent stem cells (iPSCs) [11,13]. Moreover, topologically associated domains (TADs) (three-dimensional genomic structures) play a vital role in regulating gene expression and genome size reduction. ZNF274, DNase I, H3K36 trimethylation, TSS, RNA polymerase II, specificity protein 1, and SIX homeobox 5- transcription factors are TAD boundary-related genomic elements that are crucial in the formation of chromatin structure and gene expression regulation [14]. A study on nonalcoholic fatty liver disease (NAFLD) revealed that the transcription factor-binding sites for ZNF274 are 1000-fold enriched at NAFLD-specific CpG sites [15], providing bases for treatment-induced epigenetic remodelling in humans. Nevertheless, whether ZNF274 plays a significant role in CRC prognosis and its regulatory mechanism remain elusive. This study found that IL8 stimulation increased the expression of ZNF274 in colorectal cancer cells, implying that ZNF274 might play an essential role in IL8-mediated CRC progression.

In this study, we investigated the role and molecular mechanism by which ZNF274 regulates CRC progression. We found that the expression of ZNF274 was upregulated in CRC, which was associated with poor CRC prognosis. Overexpression of ZNF274 was positively correlated with IL-8 stimulation, and ectopic expression of ZNF274 promoted the proliferation and migration of colorectal cancer cells by transactivating MRPL40. This study provides new therapeutic targets and prognostic markers for colorectal cancer.

2. Materials and methods

2.1. Cell culture

The human normal colonic epithelial cell line (NCM460) and colon cancer cell lines (HCT116, Caco-2, SW480, RKO, T84, SW620, and LoVo) were purchased from the Cell Type Bank of the Chinese Academy of Sciences and Shanghai Institute of Biochemistry and Cell Biology. The cell lines were cultured in DMEM or RPMI 1640 media supplemented with 10% fetal bovine serum according to the manufacturer's instructions. All cell culture experiments were performed in an incubator at 37 °C under 5% CO_2. For treatment with IL-8, adherent tumor cells were cultured in the presence of recombinant IL-8 (Peprotech) at the indicated concentrations for 24 h in serum-free medium.

2.2. Immunohistochemical staining

A total of 90 formalin-fixed, paraffin-embedded CRC tissues and corresponding adjacent nontumor tissues were purchased from Shanghai Outdo Biotechnology (Shanghai, China). The clinicopathological and prognostic information was obtained from the manufacturer's data sheets matched with the tissue microarray (TMA). The expression of ZNF274 protein in the TMA was analyzed by immunohistochemical assay. Briefly, CRC tissue sections were routinely deparaffinized for hydration, blocked in 3% H_2O_2 solution for 30 min, and repaired with 10% citrate antigen. Serum from normal secondary antibodies was blocked for 30 min. Then, primary antibodies against the corresponding target molecules were added dropwise at 4 °C overnight. The cells were washed with PBS, treated with biotin-labelled secondary antibody for 30 min, and rinsed with PBS. Streptomyces avidin-peroxidase complex working solution was added dropwise for 30 min. The mixture was rinsed with PBS and stained with DAB. The slides were counterstained with hematoxylin and sealed with neutral gum. Anti-ZNF274 (PA5-40385, Invitrogen, USA) was the primary antibody used in the IHC analysis in this paper.

The results of IHC staining were independently and semiquantitatively scored by two pathologists using the integral method. A five-point scale was used based on the percentage of positive cells: negative = 0 points, <10% = 1 point, 11%–50% = 2 points, 51%–80% = 3 points, and >80% = 4 points. A four-point scale was used to score the staining intensity: 0 = no positive cells, 1 represented weak positivity, 2 = moderate positivity, and 3 = strong positivity. The percentage of positive cells × staining intensity was the final score, where ≤4 indicated low expression, whereas >4 represented high expression.

2.3. Prognostic analysis of ZNF274 in CRC

Patients were divided into high- and low-expression groups based on the median value of ZNF274 expression analyzed by the immunohistochemical staining data of the tissue microarray and their corresponding clinical data. Survival probability analyses were performed using Kaplan–Meier curves (K–M). Conversely, the correlation between ZNF274 expression and clinicopathological features was analyzed by Cox regression analysis.

2.4. Western blot analysis

Total protein was extracted from cell lysates (generated using RIPA lysis buffer on ice). Protein electrophoresis, transfer to membranes and blotting were carried out according to standard protocols. Then, a gel imaging analyser was used to analyse the WB images. The primary WB antibodies used in this study were anti-ZNF274 (MA5-21159, Invitrogen, USA), MRPL40 (PA5-52265, Invitrogen), and β-actin (#4967, Cell Signaling Technology, USA).

2.5. Real-time quantitative polymerase chain reaction (RT–qPCR)

RT–qPCR was performed using the SYBR Green RT–qPCR system as previously described [16–20]. Human ACTB was used as the internal control, and the relative expression of mRNA was calculated using the $2^{-\triangle\triangle Ct}$ method. The primer sequences are listed in Supplementary Table S1.

2.6. Analysis of public databases

The GSE30364 dataset was acquired from GEO Databases. It contained transcriptional profiles of three IL-8-treated Caco-2 cells and three control samples. Differential gene expression (DGE) analysis was performed using the R package limma. The screening criteria for differentially expressed genes were log2-fold fold change $\geq$1 and false discovery rate $<$0.05. The differentially expressed genes were mapped to each node in the Gene Ontology database. DAVID (The Database for Annotation, Visualization and Integrated Discovery) was used to identify significantly enriched categories. The key differentially expressed genes upon IL-8 stimulation were mapped to the human signaling pathway network map using GO Color, a pathway mapping tool.

2.7. Plasmid transfection

The overexpression and shRNA plasmids were designed by Sangon Biotech (Shanghai, China). Plasmid transfections were performed using Lipofectamine 2000 Reagent (Invitrogen) and 1 μg of plasmid according to the manufacturer's instructions. The shRNA sequences are listed in Supplementary Table S2.

2.8. Luciferase reporter assay

Briefly, primers were designed from the genomic DNA to clone the required promoter fragments and mutant sequences, and then the sequences were inserted into the luciferase reporter plasmid (pGL3-basic). Target and control plasmids were prepared and purified for later use. The reporter gene plasmid and transcription factor expression plasmid were transfected into CRC cells. Relative fluorescence intensity was calculated and compared with the negative control. The primer sequences for the luciferase reporter assay are listed in Supplementary Table S1.

2.9. Chromatin immunoprecipitation (ChIP)-PCR assay

Cell lysates were collected after formaldehyde treatment and sonicated, and then the target protein antibody was added to bind to the target protein–DNA complex. Protein A was added, and the antibody-target protein–DNA complex was formed. The precipitated complex was washed to remove nonspecific binding antibodies. The primer sequences for the ChIP-PCR assay are listed in Supplementary Table S1.

2.10. Colony formation assay

For transfection, 5×10^2 cells were seeded in each well of 6-well plates and cultured for 14 days. The cells were fixed with 4% paraformaldehyde and incubated in 0.5% crystal violet for 10 min. Cell images were captured and counted using a colony counter and ImageJ software, respectively.

2.11. Cell counting Kit-8 (CCK-8) and cell proliferation assay

A 1×10^3 cell suspension was seeded in a 96-well plate and incubated for 1–4 h, and then the CCK-8 solution was added. The absorbance at 450 nm was measured using a microplate reader.

2.12. Transwell assay

The cells were digested with trypsin, washed twice and resuspended in serum-free basal medium. Cells in the lower chamber were suspended in DMEM (0.6 ml) supplemented with 10% FBS, whereas a 1×10^3 cell suspension with serum-free basal medium was added to the upper chamber. The cells were then incubated at 37 °C for 24 h. The cells were then fixed with 4% paraformaldehyde for 5 min and stained with 1% crystal violet for 10 min. Excess dye and water were removed from the cells, and then different fields were randomly selected and counted under the microscope.

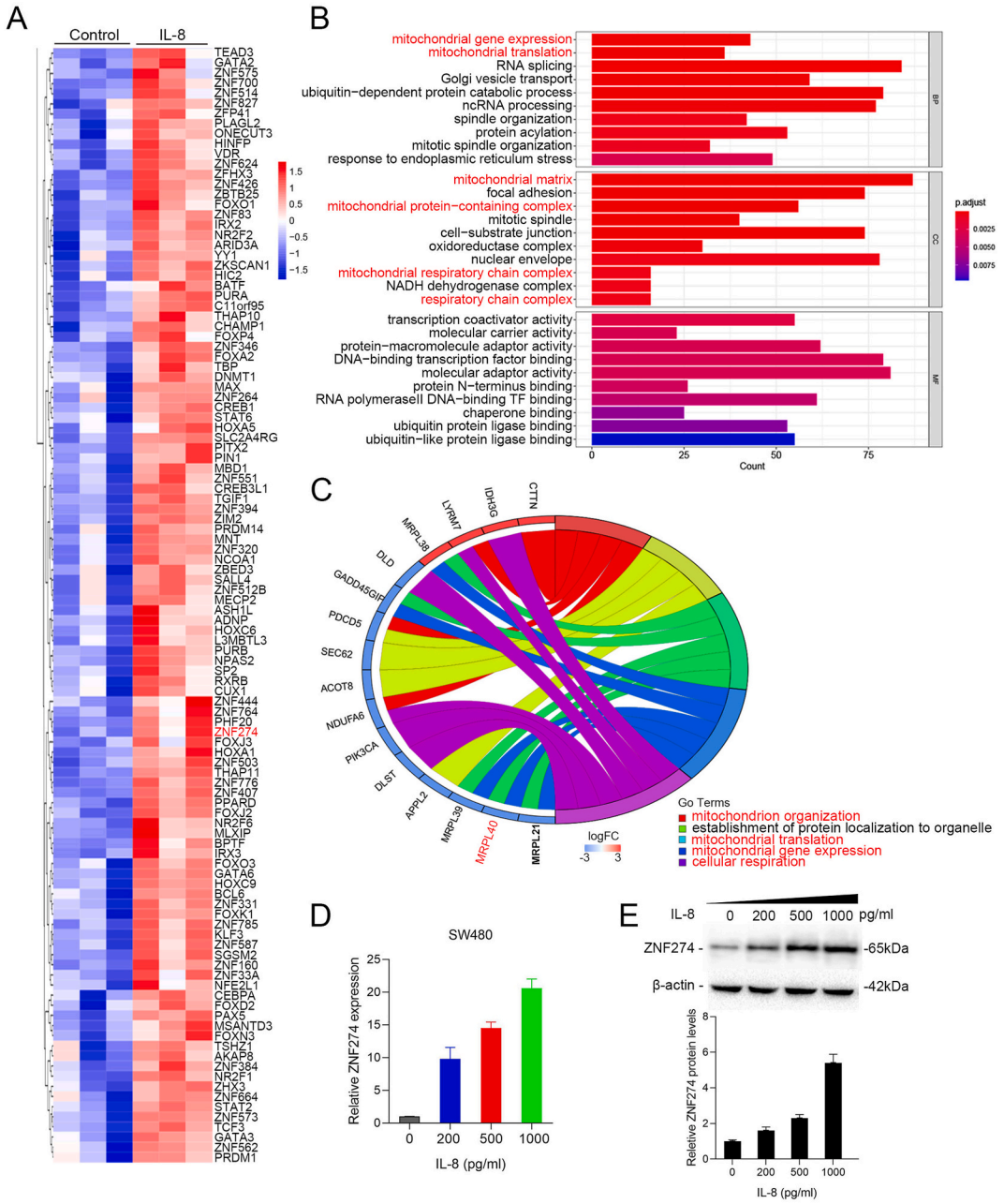

Fig. 1. The mechanism underlying IL-8-induced upregulation of ZNF274 expression. (A) Heatmap illustration of changes in differential gene expression upon IL-8 stimulation in Caco-2 cells in the GSE30364 dataset. (B) Pathway enrichment analysis of differentially expressed genes in CRC cells after IL8 stimulation. (C) Network map of the top 5 enriched signaling pathways and their interacting proteins. (D–E) qRT−PCR and western blotting analysis of the expression of ZNF274 following incubation with various concentrations of IL-8 for 24 h.

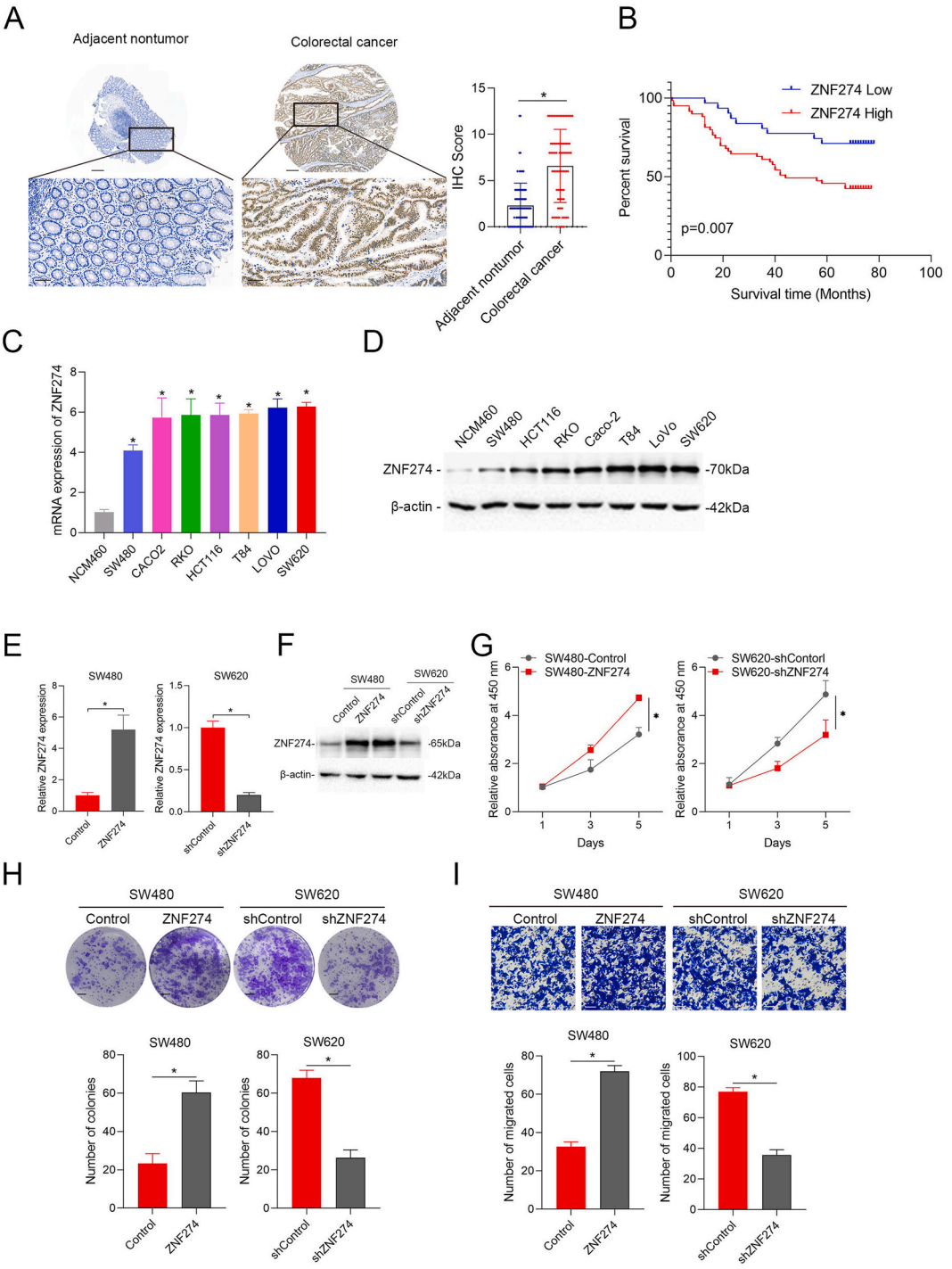

Fig. 2. Effect of ZNF274 expression on the proliferation and migration of colorectal cancer cells. (A) Immunohistochemistry staining for the expression of ZNF274 in 90 cases of CRC and 90 adjacent nontumor tissues. Scale bars represent (top) 200 μm and (bottom) 50 μm. (B) Kaplan–Meier analysis of overall survival based on ZNF274 IHC score. (C–D) qRT–PCR and western blotting for the expression of ZNF274 in a panel of CRC cell lines and normal colorectal epithelial cells NCM460. (E–F) qRT–PCR and western blotting were used to assess the efficiency of ZNF274 overexpression or knockdown. (G–H) CCK-8 and colony formation assays were used to detect cell proliferation. Scale bars = 4 mm. (I) Transwell assays were used to detect the migration abilities of SW480 and SW620 cells. Scale bars = 100 μm. Mean ± S.D., $^{*}P < 0.05$, $^{**}P < 0.01$.

2.13. Statistical analysis

R language (version 4.2) and GraphPad Prism (version 5.0) statistical software were used to analyse the experimental data. The experimental data are expressed as the mean ± standard deviation (x±SD).

3. Result

3.1. IL-8 stimulation increases ZNF274 expression and activates mitochondrial gene expression

Interleukin-8 (IL-8, also known as chemokine CXCL8) is a cytokine secreted by macrophages and epithelial cells [3]. IL-8 is dysregulated in various tumor cells, and it enhances the chemotaxis of human neutrophils and promotes the dedifferentiation, angiogenesis, invasion, and metastasis of tumor cells [3]. Recent studies have closely linked zinc finger protein (ZNF) family proteins to human cancer. ZNF transcription factors are broadly involved in tumor development and progression. Interestingly, 28 ZNF genes were upregulated following IL-8 stimulation (Fig. 1A). Furthermore, we validated the expression of differentially expressed ZNF genes using qRT−PCR. The results showed that ZNF274 had the greatest expression pattern changes during treatment with IL-8 (Supplementary Figure 1); therefore, we focused on ZNF274 for subsequent analysis.

Moreover, pathway analysis showed that IL-8 influenced mitochondrial-related bioprocesses and cellular components, including mitochondrial gene expression, mitochondrial translation, mitochondrial matrix, mitochondrial protein−containing complex, mitochondrial respiratory chain complex I, and respiratory chain complex I (Fig. 1B). In addition, transcriptional regulatory activities, such as transcription coactivator activity and DNA−binding transcription factor binding, were activated (Fig. 1B). The network map of the top 5 enriched signaling pathways and their interacting genes are shown in Fig. 1C. Critical IL-8-related mitochondrial functional genes, including MRPL38, MRPL40, GADD45GIP, PDCD5, ACOT8, and NDUFA6, were identified (Fig. 1C).

In addition, CRC cells were exposed to different concentrations of IL-8 to examine the effect of IL-8 on ZNF274 expression in CRC cells. qRT−PCR and Western blot analysis showed that ZNF274 expression increased with IL8 concentration (Fig. 1D and E). This finding suggests that IL-8 induces the expression of ZNF274. Taken together, these findings imply that mitochondrial gene expression is critical for IL-8-mediated CRC progression.

3.2. ZNF274 is overexpressed in CRC tissues and cells and is associated with poor prognosis

Immunohistochemical staining was performed on a CRC tissue microarray to explore the clinical significance of ZNF274 in CRC. The results showed that ZNF274 expression was significantly higher in CRC tissues than in adjacent nontumor tissues (Fig. 2A). K−M survival analysis revealed that CRC patients with elevated ZNF274 expression had shorter survival periods and worse prognoses (Fig. 2B). Overexpression of ZNF274 strongly correlated with larger tumor size, more invasion and lymph node metastasis, and relatively advanced AJCC stage (Table 1). Furthermore, PCR and western blotting were used to detect ZNF274 expression in CRC cell lines, and the results showed that ZNF274 expression was upregulated in CRC cell lines, including SW480, HCT116, RKO, CACO-2, T84, LoVo, and SW620, compared with the normal cell line NCM460 (Fig. 2C and D). In addition, the expression of ZNF274 was higher in highly malignant CRC cell lines (SW620, LoVo) than in less malignant CRC cell lines (SW480, Caco2) (Fig. 2C and D). Based on these results, ZNF274 expression is upregulated in CRC and might play a significant role in CRC progression.

Table 1
Correlation between ZNF274 expression and clinicopathological characteristics in 90 cases of human CRC tissues.

Clinicopathological variables		ZNF274 expression		p Value
		Low (n = 31)	High (n = 59)	
Age		65.00 (11.815)	65.78 (13.749)	0.789
Sex	female	18	25	0.157
	male	13	34	
Tumor location	right colon	18	29	0.421
	left colon	13	30	
Tumor size	< 5 cm	20	18	**0.002**
	≥5 cm	11	41	
Tumor invasion	T1	1	0	**<0.001**
	T2	8	1	
	T3	18	34	
	T4	4	24	
Lymph node metastasis	N0	29	32	**0.001**
	N1	2	19	
	N2	0	8	
Distant metastasis	absent	31	56	0.202
	present	0	3	
AJCC stage	Stage I	9	1	**<0.001**
	Stage II	20	29	
	Stage III	2	6	
	Stage IV	0	3	

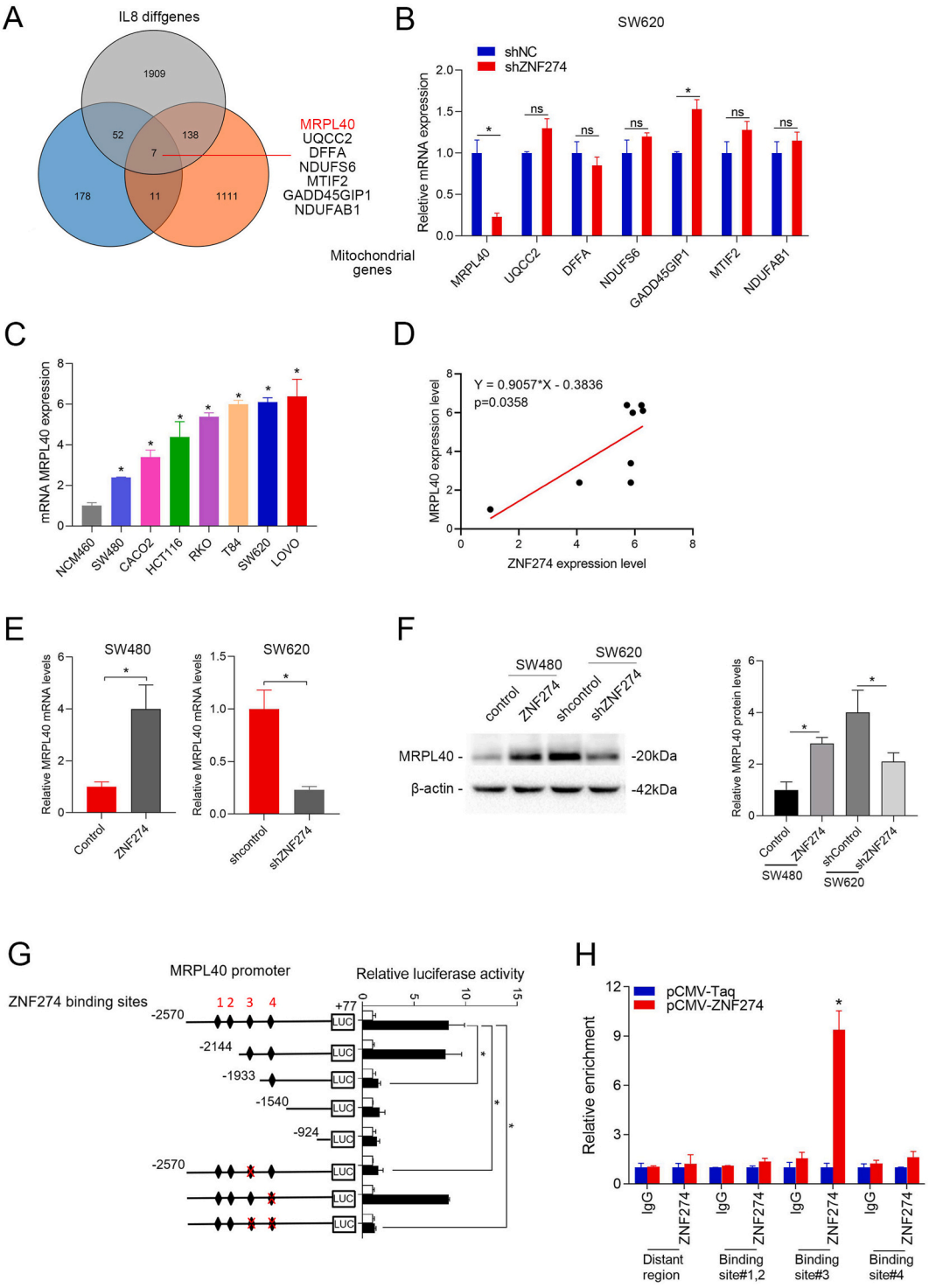

Fig. 3. Identification of MRPL40 as a downstream target gene of ZNF274. (A) Venn diagram depicting the intersection of genes between different categories. (B) Effect of ZNF274 knockdown on the expression of putative target genes in SW620-shZNF274 cells. Mean ± S.D., $*P < 0.05$, $**P < 0.01$. (C) qRT−PCR assay for the expression of MRPL40 in a panel of CRC cell lines and normal colorectal epithelial cells (NCM460). (D) The correlation between the expression levels of ZNF274 and MLRP40. (E–F) MRPL40 expression was measured by qRT−PCR and western blotting after ZNF274 ectopic expression or knockdown. (G) Wild-type, truncated, and mutant MRPL40-luciferase reporters were transfected with ZNF274, followed by the determination of MRPL40 promoter activity. (H) SW480 cells overexpressing the vector or the MRPL40 plasmid were incubated in a ChIP-PCR assay to detect enrichment of ZNF274 in the MRPL40 promoter. Mean ± s.d., $*P < 0.05$, $**P < 0.01$.

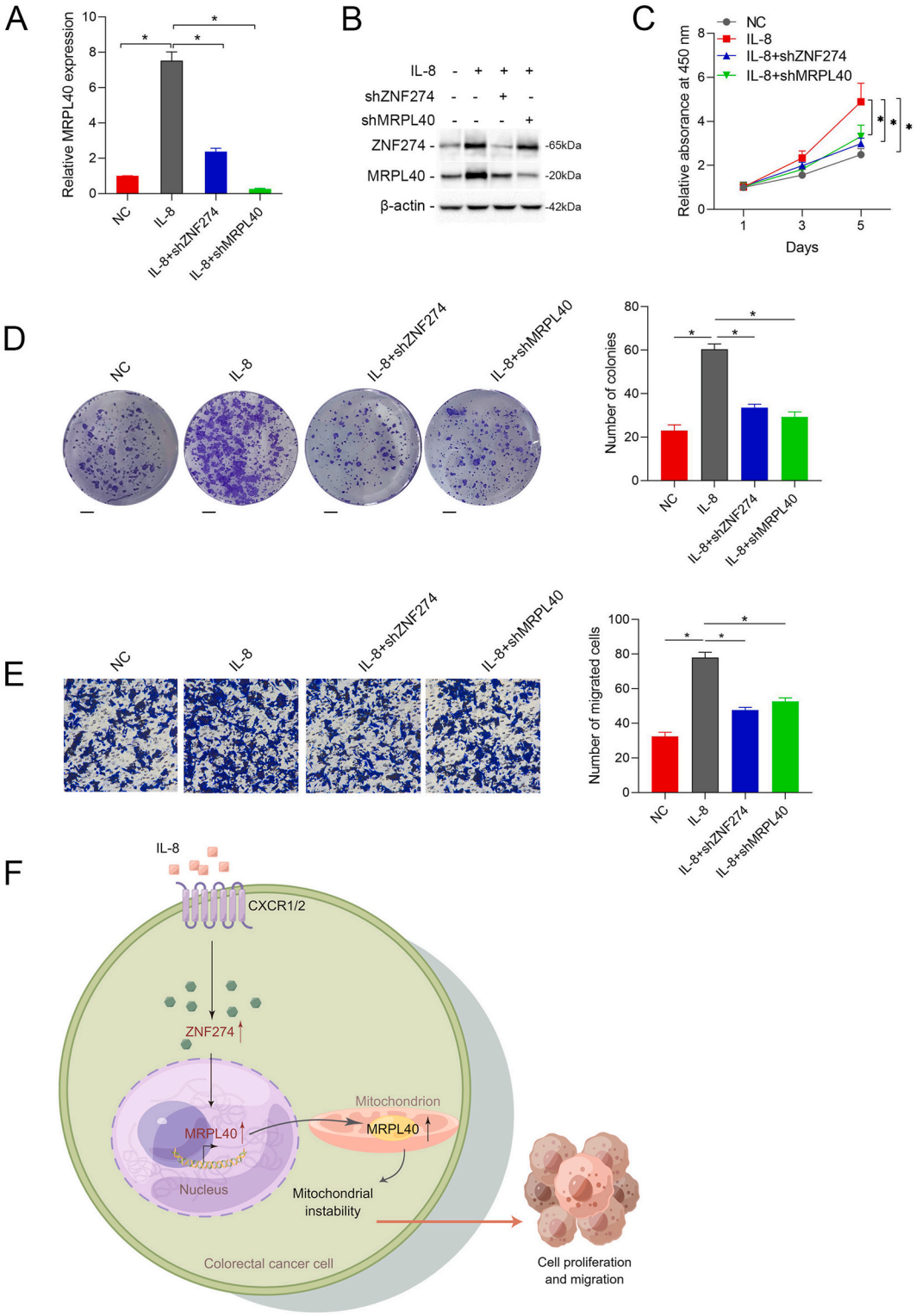

Fig. 4. Effects of ZNF274 and MRPL40 on IL8-mediated proliferation and migration of colorectal cancer. (A–B) MRPL40 expression was evaluated following IL-8 (500 pg/ml, 24 h) stimulation in SW480 cells knocked down for ZNF274 or MRPL40. (C–D) Cell proliferation was detected by CCK-8 and colony formation assays. Scale bars = 4 mm. (E) Transwell assays were used to detect cell migration capabilities. Scale bars = 100 μm. Mean ± s.d., $^{*}P < 0.05$, $^{**}P < 0.01$. (F) Graphical abstract. IL-8-mediated overexpression of ZNF274 promotes the proliferation and migration of colorectal cancer cells through the transactivation of MRPL40.

3.3. ZNF274 promotes the proliferation and migration of colorectal cancer cells

To clarify the function of ZNF274 in CRC progression, we chose the SW480 cell line, which has relatively low ZNF274 expression and low malignancy, to overexpress ZNF274 for gain-of-function (Fig. 2E and F, left). In contrast, we chose the SW620 cell line, which has relatively high ZNF274 expression and high malignancy, to knock down ZNF274 for loss-of-function (Fig. 2E and F, right). The CCK-8 assay showed that overexpression of ZNF274 significantly promoted the proliferation of CRC cells, whereas ZNF274 knockdown inhibited cell proliferation (Fig. 2G). In addition, ectopic expression of ZNF274 significantly increased the colony formation ability of CRC cells; however, ZNF274 knockdown exerted the opposite effect (Fig. 2H). Transwell assays showed that upregulated ZNF274 expression promoted the migration of CRC cells, while ZNF274 knockdown significantly inhibited the migration of CRC cells (Fig. 2I). These results indicate that ZNF274 promotes the proliferation and migration of CRC cells.

3.4. ZNF274 transcriptionally activates mitochondrial large ribosomal protein L40 (MRPL40)

First, 1268 genes potentially targeted by ZNF274 were identified from six transcription factor datasets (CHEA, ENCODE, JASPAR, MotifMap, TRANSFAC, and TRRUST) to explore the effect of ZNF274 in CRC. The results revealed that seven genes, including UQCC2, MRPL40, GADD45GIP1, DFFA, NDUFS6, MTIF2, and NDUFAB1, were shared in the intersection of IL-8-related differentially expressed genes, ZNF274 target genes, and mitochondria-related gene sets (Fig. 3A). Mitochondrial large ribosomal protein L40 (MRPL40), which plays roles in mitochondrial energy metabolism, mitochondrial translation, and protein metabolism [21], was significantly upregulated after ZNF274 knockdown (Fig. 3B). As a result, MRPL40 was selected for further verification.

PCR analysis showed that the expression of MRPL40 was significantly upregulated in CRC cell lines (Fig. 3C), and there was a strong positive correlation between ZNF274 and MRPL40 expression (Fig. 3D). To determine whether MRPL40 is a direct target gene of ZNF274, MRPL40 expression was detected in ZNF274-overexpressing (SW480-ZNF274) and ZNF274-knockdown (SW480-shZNF274) cells. MRPL40 expression significantly increased with ZNF274 overexpression and decreased with ZNF274 knockdown (Fig. 3E and F). A Jasper analysis of the MRPL40 promoter revealed four ZNF274 binding sites, and a dual-luciferase reporter assay was used to determine whether ZNF274 regulates MRPL40 transcription (Fig. 3G). In the truncation experiment, ZNF274 transcriptionally activated MRPL40 depending on binding site 3 (Fig. 3G). In addition, mutagenesis experiments showed that mutation of binding site 3 inhibited the transactivation of MRPL40 by ZNF274 (Fig. 3G). Furthermore, ChIP-PCR analyses confirmed that ZNF274 binds directly to binding site −3 on the MRPL40 promoter. (Fig. 3H). These results show that ZNF274 is a direct target gene that transactivates MRPL40 expression by binding to the promoter region of MRPL40.

3.5. ZNF274 and MRPL40 are essential for IL8-mediated colorectal cancer cell proliferation and migration

To further understand the role of ZNF274, we explored whether ZNF274 and its target gene MRPL40 contribute to IL8-related CRC progression. ZNF274 and MRPL40 were knocked down in cells stimulated with IL-8. The expression of MRPL40 was highly induced by IL-8, whereas ZNF274 knockdown inhibited the expression of MRPL40 induced by IL-8 (Fig. 4A and B). In functional rescue experiments, ZNF274 or MRPL40 knockdown significantly reversed the IL-8-induced proliferation of CRC cells (Fig. 4C). Accordingly, IL-8 stimulation increased colony formation, whereas knockdown of ZNF274 and MRPL40 reduced cell viability and colony formation in CRC cells (Fig. 4C and D). Moreover, Transwell migration assays showed that IL-8 enhanced the migration ability of CRC cells, while ZNF274 or MRPL40 knockdown significantly inhibited IL-8-mediated migration (Fig. 4E). These results suggest that ZNF274 and its target gene MRPL40 are essential for the IL8-promoting proliferation and migration of CRC.

4. Discussion

Understanding the molecular regulatory mechanism in the CRC process and identifying novel therapeutic targets for CRC could help improve patient prognoses. This study provides promising new therapeutic targets for CRC treatment. We found that the expression of ZNF274 was significantly higher in CRC tissues and cells than in their nontumor counterparts. A higher level of ZNF274 was associated with poor prognosis and specific clinicopathological characteristics in CRC patients. The results are in line with the findings in a glioma study that ZNF274 expression negatively correlates with ATRX expression and plays a vital role in rapidly progressing aggressive tumors [22]. Therefore, ZNF274 can be a potential oncogene and a prognostic factor in human cancer.

In general, transcription factors are responsible for modulating downstream target genes through transcriptional regulation [23]. ZNF274 has mainly been discussed in the context of neurobehavioral disorders [11,13,24–26]. ZNF274 is reported to transcriptionally repress SNORD116 expression, and ZNF274 knockdown reactivates SNORD116 maternal expression in Prader-Willi syndrome (PWS)-induced pluripotent stem cells (iPSCs) [11,26]. Interfering with the binding of ZNF274 to the maternal SNORD116 promoter is potentially a treatment strategy for PWS [11,13,25]. ChIP-sequencing analysis showed that SETDB1, KAP1, and H3K9me3 are colocalized with ZNF274's binding site at the 3′ end of zinc finger genes [12]. The KRAB domains of ZNF274 exhibited transcriptional repressor activity when tested in GAL4 fusion protein assays. Furthermore, a topologically associated domain (TAD) is a 3D genomic structure that regulates gene expression by interacting with its surroundings [14]. ZNF274, with DNase I, H3K36 trimethylation, TDD, RNA polymerase II, and specific protein- 1, were identified as TAD boundaries that designated gene expression and chromosome remodelling [14]. Studies have demonstrated that ZNF274 plays a vital role in regulating gene expression and forming chromatin structures. To date, ZNF274 targeting has not been applied in CRC. Our findings showed that ZNF274 significantly promotes CRC cell proliferation and migration, demonstrating an oncogenic role of ZNF274 in CRC progression. We also found that ZNF274

transactivated MRPL40, and downregulating ZNF274 and MRPL40 significantly reversed CRC cell migration and proliferation induced by IL-8. These findings suggest that ZNF274 plays an essential role in the progression of CRC. Our subsequent study will explore the molecular mechanism of ZNF274 expression in relation to IL-8.

Mitochondria play critical roles in the maintenance of cellular homeostasis [27]. Mitochondrial ribosomal protein L40 (MRPL40) is a primary regulator of mitochondrial energy metabolism, mitochondrial translation, and protein metabolism [28,29]. MRPL40 deficiency leads to mitochondrial calcium dysregulation, reducing synaptic plasticity and cognitive deficits [28,30,31]. In human cancer, MRPL40 expression is significantly elevated in breast cancer cells and is related to the mitochondrial translation of proteins in the OXPHOS complex [21]. The above studies revealed that MRPL40 is essential for mitochondrial homeostasis and tumor progression. However, the role and mechanism of MRPL40 in CRC need further investigation. This study, for the first time, reports that MRPL40 is a direct target gene of ZNF274 in CRC. We found that ZNF274 transcriptionally activates MRPL40, and a positive correlation exists between MRPL40 expression. ZNF274 and MRPL40 are essential for IL8-mediated colorectal cancer proliferation and migration. These observations demonstrate that ZNF274 is functionally associated with MRPL40.

More importantly, this study implies that the ZNF274-MRPL40 axis can offer a promising therapeutic target for CRC. Currently, there are no targeted pharmacologic small molecule inhibitors or antibodies against ZNF274 or MRPL40. Despite this, promising approaches include nanoparticle delivery platforms that can ultimately result in both controlled delivery and tissue-specific targeting. The codelivery nanovehicles can be used for therapeutic delivery of a broad spectrum of siRNAs or miRNAs. The development of targeted drug delivery systems for ZNF274 and MRPL40 has important clinical application value.

There are several limitations of this study. First, the current study suffers from the limitation that the results are obtained mainly from cell culture and lack verification by animal experiments. These experiments could be carried out with in vivo experiments to better ensure the reliability of the results. Another limitation of this study is that the clinical sample number was small, and more patients should be included in future studies.

5. Conclusion

This study demonstrates that ZNF274 is highly expressed in CRC tissues and cell lines, and high ZNF274 expression correlates with poor prognosis in CRC patients. ZNF274 plays an oncogenic role in regulating the proliferation and migration of CRC cells. ZNF274 transactivates MRPL40 expression by directly binding to its promoter region, and the ZNF274-MRPL40 axis is essential for IL8-promoted CRC proliferation and migration. Meanwhile, experiments with in vivo animals need to be performed. Additionally, it is important to investigate the molecular mechanisms involved in regulating ZNF274 by IL-8.

Author contribution statement

Feng Du: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Shengtao Zhu: Conceived and designed the experiments; Wrote the paper.

Nan Zhang: Performed the experiments.

Yijun Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xu Ji; Junxuan Xu: Analyzed and interpreted the data.

Peng Li: Analyzed and interpreted the data; Wrote the paper.

Tingting Ning; Si-an Xie: Contributed reagents, materials, analysis tools or data.

Si Liu: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19046.

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