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Elevated N-Myc downstream-regulated gene 3 expression indicates poor survival in epithelial ovarian cancer

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Abstract

N-Myc downstream-regulated gene 3 (NDRG3), a member of the NDRG family, plays an important role in the development, progression, invasiveness, and metastasis of multiple tumor types. This study focuses on NDRG3 expression in epithelial ovarian cancer (EOC) and the correlation between NDRG3 expression and prognostic indicators. First, the LinkedOmics database was used to analyze the expression of genes associated with NDRG3, and then gene ontology and Kyoto encyclopedia of genes and genomes (KEGG) functional enrichment analyses and methylation analysis of NDRG3-related genes were performed to identify co-expressed genes. A protein–protein interaction network was constructed using the STRING database. Subsequently, quantitative polymerase chain reaction was performed to determine the mRNA expression level of NDRG3 in 22 fresh EOC tissue samples. In addition, immunohistochemistry was performed to detect the expression of NDRG3 protein in 110 EOC microarray samples. Cox regression and Kaplan–Meier survival analyses were performed to assess the prognostic value of NDRG3. Bioinformatics analysis showed that NDRG3 had a broad impact on the transcriptome and that genes that were co-expressed with NDRG3 were primarily involved in organ- or tissue-specific immune response, response to chemokine, interleukin-1 production, and other related pathways. The KEGG pathway analysis suggested that genes co-expressed with NDRG3 were also enriched in signaling pathways, including the interleukin-17 signaling pathway. The mRNA expression levels of NDRG3 were significantly higher in EOC tissues than in paracancerous nontumor tissues ($P < .01$). NDRG3 expression in EOC was correlated with distant metastasis ($P = .02$), tumor–node–metastasis stage ($P = .03$), and patient prognosis ($P = .01$). Moreover, the disease-free survival and overall survival times of EOC patients decreased with increasing NDRG3 expression. High NDRG3 expression and lymph node metastasis were identified as independent prognostic factors in 110 EOC patients. NDRG3 plays a key role in ovarian cancer progression. High NDRG3 expression is correlated with multiple clinicopathologic features of EOC and may be an indicator of a poor prognosis in EOC.

Abbreviations: 95% CI = 95% confidence interval, AJCC = the American Joint Committee on Cancer, CA125 = cancer antigen 125, DFS = disease-free survival, EOC = epithelial ovarian cancer, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, GO = gene ontology, GSEA = gene-set enrichment analysis, HCC = hepatocellular carcinoma, HR = hazard ratio, IL-17 = interleukin-17IHC = immunohistochemistry, KEGG = Kyoto encyclopedia of genes and genomes, LSCC = laryngeal squamous cell carcinoma, NADH = nicotinamide adenine dinucleotide hydrideNDRG3 = N-Myc downstream-regulated gene 3, OC = ovarian cancer, OS = overall survival, PPI = protein–protein interaction, qPCR = quantitative polymerase chain reaction, TCGA = the cancer genome atlas, TMA = tissue microarray, TNM = tumor node metastasis.

Keywords: epithelial ovarian cancer, immunohistochemistry, LinkedOmics, NDRG3, prognosis

CS and LS contributed to this article equally.

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Informed consent was obtained from all patients.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

This study was reviewed and approved by the Ethics Committee of Nanjing Pukou People's Hospital, Nanjing, People's Republic of China.

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1. Introduction

Ovarian cancer is a prevalent gynecological malignancy, accounting for 3.4% and 4.7% of gynecological malignancies and related deaths, respectively.[1] In China, as elsewhere, OC seriously endangers women's health, and its morbidity and mortality rank in the top 10 of all gynecological malignancies.[2] Compared with other tumors, OC has complex histopathological types, including epithelial ovarian cancer (EOC), germ cell malignancies, sex cord stromal tumors, and others. EOC, which is the predominant type of OC, accounts for approximately 90% of cases and has various histopathological subtypes, and of these, high-grade serous carcinoma is the most common in clinical practice.[3] EOC is characterized by late detection and high mortality, and most patients are diagnosed at an advanced stage. The average 5-year survival rate of EOC patients is approximately 45.6%, that of patients who are diagnosed early can exceed 70%, and that of patients diagnosed late is only 35%.[4] Although surgical treatment, chemotherapy, radiotherapy, targeted therapy, and immunotherapy have improved substantially in recent years, the therapeutic effect in patients with advanced cancer is still poor, and no good prevention or treatment measures have been established for tumor recurrence and metastasis.[5–7] In the diagnosis and treatment of EOC, tumor markers such as cancer antigen 125 (CA125) have been widely used for clinical diagnosis and tumor burden assessment. Clinically, however, the discovery of new tumor markers for early diagnosis, precision treatment, and prognostic evaluation remains an urgent task.[8,9]

The N-Myc downstream-regulated gene (NDRG) family is a subset of the α/β-hydrolase superfamily and is repressed by Myc expression.[10–12] The NDRG family includes NDRG1, NDRG2, NDRG3, and NDRG4, and of these, NDRG3 is a crucial member that is associated with important physiological functions, including cell proliferation, differentiation, and embryonic development.[13,14] NDRG3 is also associated with the pathogenesis of several diseases. NDRG3 also mediates lactate signaling during hypoxia. Studies have shown that under hypoxia, stabilized NDRG3 can promote angiogenesis and cell growth through the Raf-ERK pathway.[15,16] Yao et al[17] found that the let-7f/NDRG3 pathway might be a target for the treatment of ischemic stroke. Recent studies have indicated that NDRG3 not only participates in tumor progression but is also related to tumor metastasis. Liu et al[18] found that abnormal NDRG3 expression was correlated with the histological grade of prostate cancer and was an important factor for the poor prognosis of gastric cancer and that decreased NDRG3 protein expression can contribute to an improved prognosis. Yin et al[19] found that high expression of NDRG3 was associated with poor survival outcomes in hepatocellular carcinoma (HCC). Ma et al[20] found that high NDRG3 protein expression was correlated with a poor prognosis in laryngeal squamous cell carcinoma (LSCC) patients and that high NDRG3 expression was associated with lymph node metastasis in LSCC. Jing et al[21] also reached a similar conclusion in HCC and reported that high NDRG3 expression was an important tumor marker for a poor prognosis in HCC patients.

The above results suggest that as an important oncogene, NDRG3 is a tumor marker correlated with the clinicopathological features and prognosis of multiple malignancies. However, the roles of NDRG3 in EOC, a common malignant tumor seen in obstetrics and gynecology, have not been reported. In this study, we investigated NDRG3 mRNA and protein expression in EOC cells and paracancerous tissue, the relationships between NDRG3 expression and the clinicopathological features of EOC, and the value of NDRG3 expression in the prognostic prediction of EOC patients. In addition, the possible mechanisms were explored through a bioinformatics analysis.

2. Materials and methods

2.1. Tissue specimens of EOC patients

The data of 110 EOC patients treated at Jiangsu Cancer Hospital from 2005 to 2015 were collected. All cases were reviewed by 2 senior pathologists. We also collected the clinical and pathological data, including age, tumor size, histological type, degree of differentiation, and lymph node and distant metastases. Tumor stage was determined according to the 7th edition of the American Joint Committee on Cancer staging manual. This study was approved by the relevant ethics committees of the Nanjing Pukou People's Hospital, and informed consent was obtained from all patients.

2.2. LinkedOmics database analysis

The LinkedOmics database[22] is a web-based database platform that contains data on 32 cancer types. In this study, the LinkedOmics ovarian cancer dataset was used to analyze the correlation and functional enrichment of NDRG3. The RNAseq data type and HiSeq RNA platform containing 303 cases of ovarian cancer were selected, and the LinkedOmics LinkFinder module was used to investigate differentially expressed genes associated with NDRG3 in the Cancer Genome Atlas. Pearson correlation coefficients were used for statistical analysis. Subsequently, we performed Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) functional enrichment analyses on the differentially expressed mRNAs according to NDRG3 expression using the LinkInterpreter module in the LinkedOmics bioinformatics database. GO enrichment analysis results were divided into 3 separate sub-ontologies: biological processes, cellular components, and molecular functions. Next, genes associated with NDRG3 methylation were screened (false discovery rate [FDR] < .01) and were also subjected to GO and KEGG analyses. The significance threshold was FDR < .05, and 500 simulations were performed.

2.3. Protein–protein interactions network construction

The STRING database (version 11.0, https://string-db.org/) is a public data resource. In this study, the STRING database was used to construct a PPI network to predict the potential relationship of protein–protein interactions (PPIs). The 10 interacting proteins most closely related to NDRG3 were predicted, and the constructed PPIs were visualized using the website.

2.4. One-step reverse transcription–quantitative polymerase chain reaction detection

We collected fresh tumor tissues and paracancerous tissues from 22 of the EOC patients referenced above. The operating procedures for quantitative polymerase chain reaction (qPCR), including RNA extraction, amplification, and data analysis, were similar to those described in the literature.[23,24] The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA level was measured to standardize the measurements of the target genes. The NDRG3 primers used were as follows: forward primer: 5'-CCA GGA CTT TGACTG TCA GGA-3'; reverse primer: 5'- AGT GCTGGG TGA. TCT CTT GC-3'. The GAPDH primers used were as follows: forward primer: 5'-TGC ACC ACC AAC TGC TTA GC-3'; reverse primer: 3'-GGC ATG GAC TGT1 GGT CAT GAG-5'.[21] The 1-step reverse transcription–qPCR procedure was as follows: reverse transcription at 42 °C for 30 minutes, double-stranded DNA predenaturation at 94 °C for 2 minutes, and 35 PCR cycles at 95 °C for 20 seconds, 56 °C for 20 seconds, and 72 °C for 30 seconds.

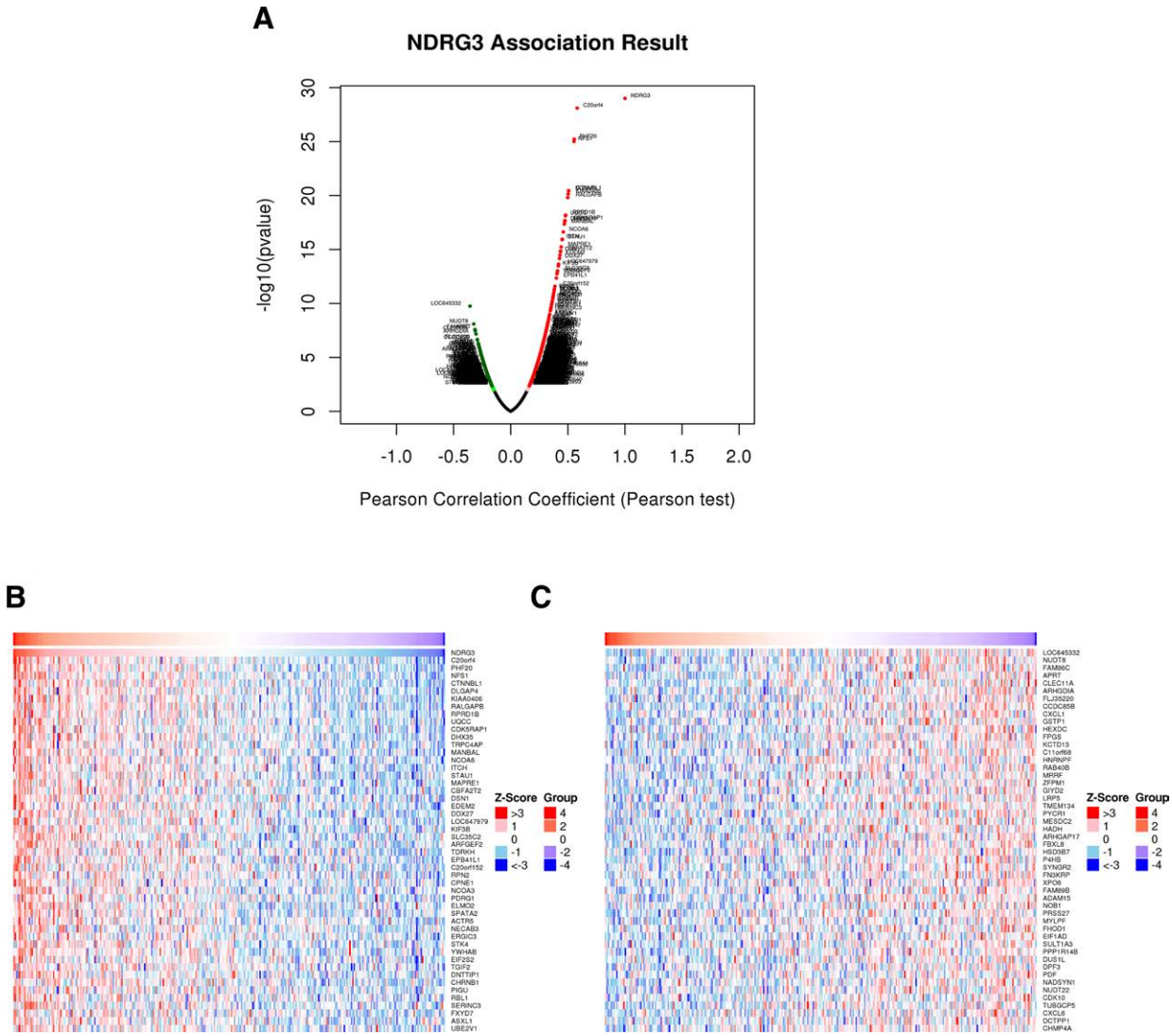

Figure 1. Differentially expressed genes associated with NDRG3 in EOC according to LinkedOmics analysis. (A) The correlation of NDRG3 with differentially expressed genes in EOC was analyzed using the Pearson correlation test. (B and C) Heat map showing the top 50 genes that were positively or negatively correlated with NDRG3 in EOC. Red represents positively correlated genes, and green represents negatively correlated genes. EOC = epithelial ovarian cancer, NDRG3 = N-Myc downstream-regulated gene 3.

2.5. Tissue microarray preparation and immunohistochemistry analysis

The tumor tissues and paracancerous tissues of 110 EOC patients were used to generate TMAs. All specimens were fixed in 10% neutral-buffered formalin and then paraffin-embedded. Round tissue blocks 1.5 mm in diameter were obtained from each patient's paraffin tissue block. The round tissue blocks of several patients were used to construct 1 large paraffin tissue block. Then, 4-μm-thick slices were prepared from the large tissue block constructed for the TMA. The NDRG3 monoclonal antibody was purchased from Abcam (Cambridge) (NDRG3, 1:200), and the secondary antibody was purchased from Dako Cytomation (Carpinteria). Immunohistochemistry (IHC) scoring consisted of 2 parts and included the positivity level and the percentage of positive cells. Tumor cell positivity levels were scored as follows: negative, 0; weakly positive, 1; moderately positive, 2; and strongly positive, 3. The percentages of positive cells were scored as follows: 0% to 20%, 1; 21% to 50%, 2; 51% to 75%, 3; and 76% to 100%, 4. IHC scoring was performed by 2 pathologists. A sum of 2 scores greater than or equal to 5 indicated high expression, while a sum <5 indicated low expression.

2.6. Statistical analysis

STATA 14.0 (Stata Corporation, College Station) was used for statistical analysis. The Wilcoxon test was used to analyze NDRG3 protein expression. The chi-squared test was used to analyze correlations between NDRG3 protein expression and the clinicopathological features of EOC. Kaplan–Meier analysis (log-rank test) was used to explore the relationship between NDRG3 and the prognosis of EOC patients. The Cox proportional hazard model was adopted for univariate and multivariate survival analyses. $P < .05$ was considered statistically significant.

3. Results

3.1. Correlation and functional enrichment analyses of differentially expressed genes associated with NDRG3 expression in EOC

The results of mRNA sequencing data of 303 EOC patients were analyzed using the LinkedOmics functional module. As shown in the volcano plot (Fig. 1A), 11,220 genes were positively correlated and 8812 genes were negatively correlated with NDRG3 expression. The heat maps (Fig. 1B and C)

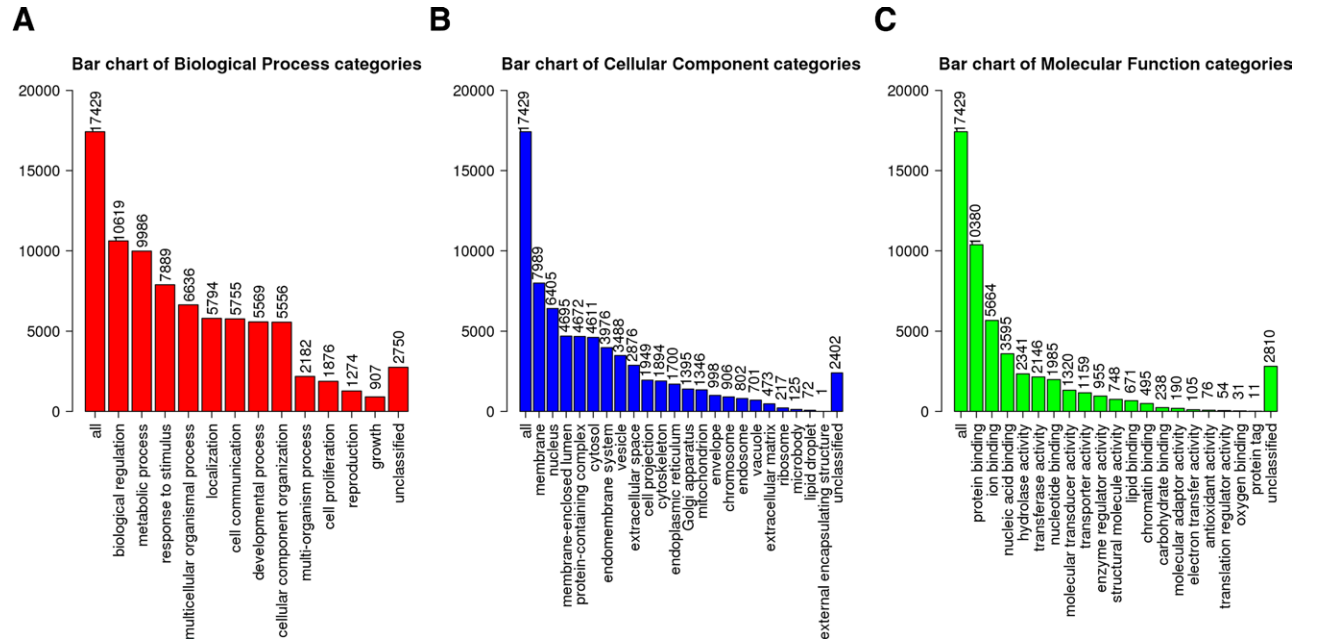

Figure 2. Gene ontology term analysis of NDRG3 expression-related genes in EOC. In terms of (A) biological process, (B) cell composition, and (C) molecular function. EOC = epithelial ovarian cancer, NDRG3 = N-Myc downstream-regulated gene 3.

Table 1

Enriched GO and KEGG items.

Enriched category	Description	Count	NES	*P*-value	FDR
Biological process					
GO:0002251	Organ-or tissue-specific immune response	25	−2.047	<.001	0.01
GO:1990868	Response to chemokine	86	−2.175	<.001	0.01
GO:0032612	Interleukin-1 production	86	−1.864	<.001	<0.05
GO:0001773	Myeloid dendritic cell activation	27	−1.842	<.001	<0.05
GO:0070671	Response to interleukin-12	48	−1.863	<.001	<0.05
Cellular components					
GO:0042611	MHC protein complex	19	−2.247	<.001	<0.01
GO:0035327	Transcriptionally active chromatin	20	1.660	.01	0.05
GO:0005776	Autophagosome	84	1.809	<.001	0.05
GO:0044450	Microtubule organizing center part	150	1.671	<.001	0.05
GO:0030990	Intraciliary transport particle	26	1.682	<.05	0.06
Molecular function					
GO:0003823	Antigen binding	52	−2.111	<.001	<0.001
GO:0042287	MHC protein binding	24	−1.920	<.001	0.02
GO:0003735	Structural constituent of ribosome	153	−1.866	<.001	0.02
GO:0016684	Oxidoreductase activity, acting on peroxide as acceptor	54	−1.770	<.001	0.04
GO:0016209	Antioxidant activity	76	−1.595	<.001	0.08
KEGG pathway					
hsa04657	IL-17 signaling pathway	89	−2.042	<.001	<0.001
hsa03010	Ribosome	130	−2.119	<.001	<0.001
hsa05320	Autoimmune thyroid disease	47	−2.157	<.001	<0.001
hsa05322	Systemic lupus erythematosus	122	−2.173	<.001	<0.001
hsa05133	Pertussis	74	−2.191	<.001	<0.001

FDR = false discovery rate, IL-17 = interleukin-17, GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes, NES = normalized enrichment score.

showed the top 50 genes that were most significantly positively or negatively correlated with NDRG3, and the results suggested that NDRG3 exerted a broad influence on the transcriptome. In the LinkInterPreter module, we utilized the Select Tool for gene-set enrichment analysis. The Enrichment Analysis encompassed biological processes, cellular components, and molecular functions, as well as KEGG pathway analysis. Figure 2A–C illustrates the number of genes encompassed within various significant biological pathway categories, specifically biological regulation, metabolic process, response to stimulus, multicellular organismal process, and localization across biological processes, cellular components, and molecular functions. More specifically, as detailed in Table 1, genes associated with NDRG3 are primarily enriched in biological process terms such as organ-or tissue-specific immune response (GO:0002251), response to chemokine (GO:1990868), interleukin-1 production (GO:0032612), myeloid dendritic cell activation (GO:0001773), and response to interleukin-12 (GO:0070671). In terms of cellular components, these genes are predominantly enriched within the MHC protein complex (GO:0042611), transcriptionally active chromatin (GO:0035327), autophagosome (GO:0005776),

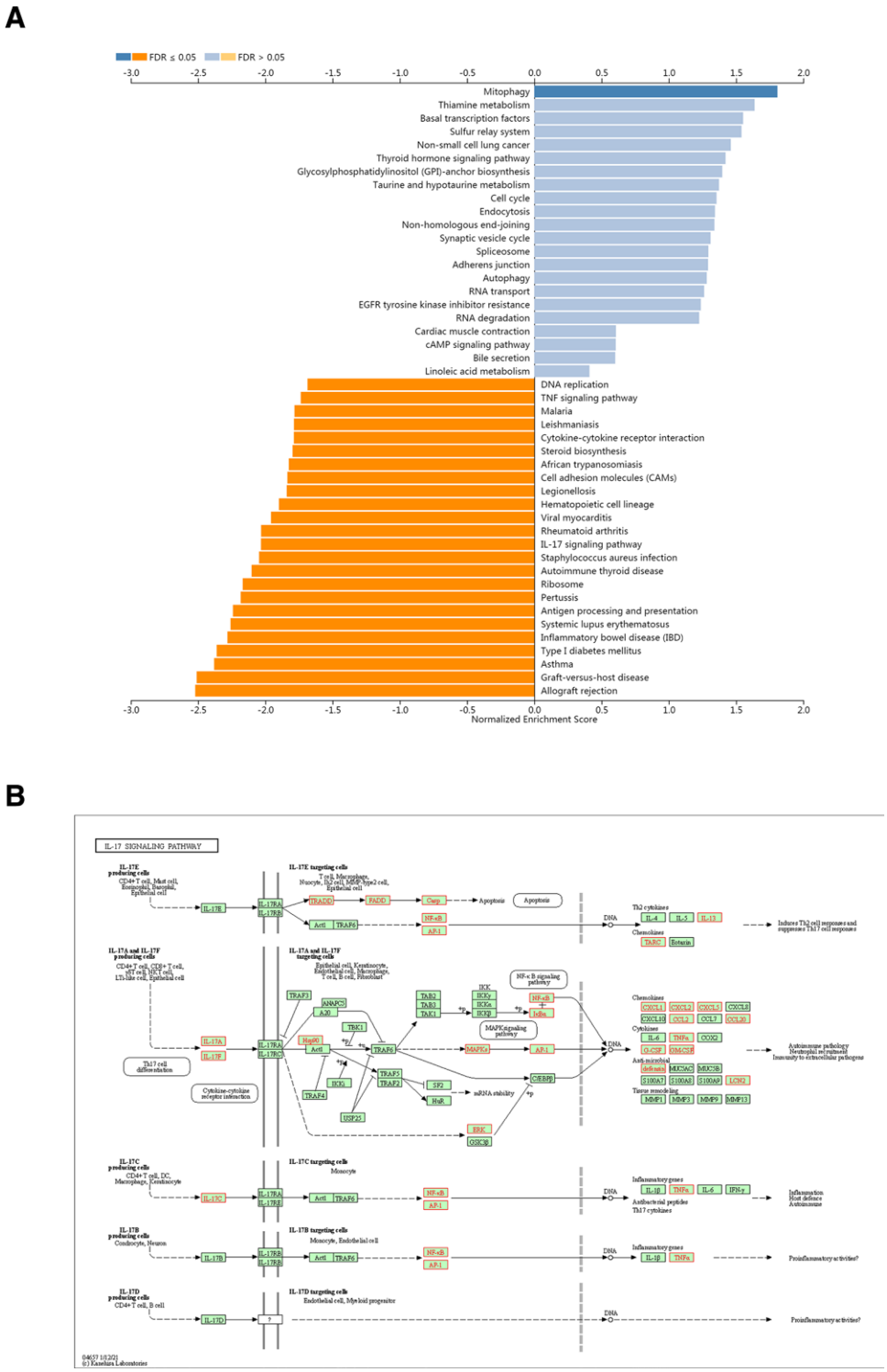

Figure 3. Kyoto encyclopedia of genes and genomes pathway enrichment analysis revealed enrichment of the IL-17 signaling pathway. (a) KEGG pathway analysis of NDRG3-related genes. (b) IL-17 signaling pathway map. IL-17 = interleukin-17, KEGG = Kyoto encyclopedia of genes and genomes, NDRG3 = N-Myc downstream-regulated gene 3.

microtubule organizing center part (GO:0044450), and intraciliary transport particle (GO:0030990). Regarding molecular functions, NDRG3-related genes show enrichment in antigen binding (GO:0003823), MHC protein binding (GO:0042287), structural constituent of ribosome (GO:0003735), oxidoreductase activity acting on peroxide as acceptor (GO:0016684), and antioxidant activity (GO:0016209). KEGG pathway enrichment analysis (Fig. 3A and Table 1) reveals that NDRG3-related genes are prominently enriched in biological pathways including interleukin-17 (IL-17) signaling pathway (hsa04657), ribosome (hsa03010), autoimmune thyroid disease (hsa05320), systemic lupus erythematosus (hsa05322), and pertussis (hsa05133). The detailed map of the key IL-17 signaling pathway is presented in Figure 3B, while the major KEGG pathway enrichment plot is shown in Figure 4.

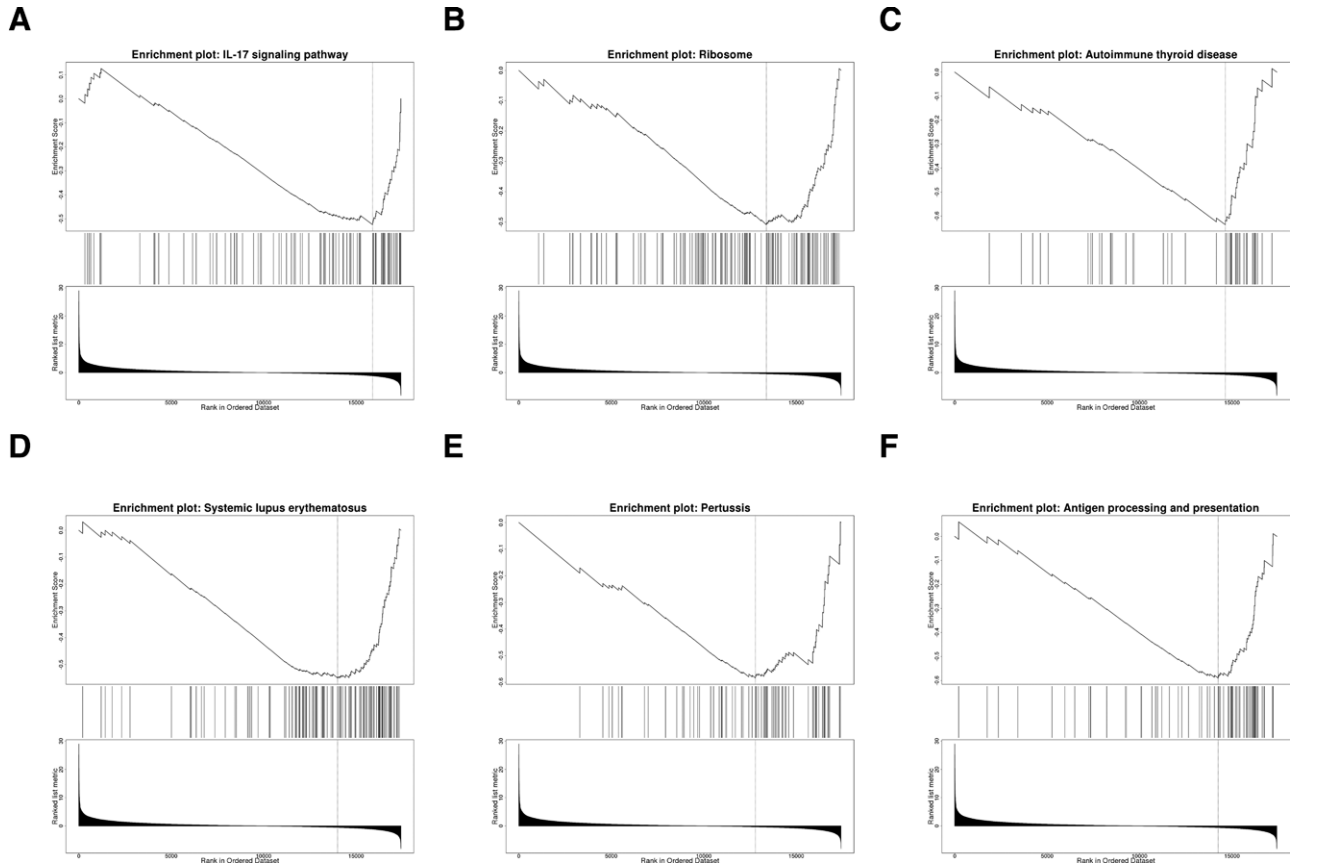

Figure 4. Gene-set enrichment analysis analysis of genes related to NDRG3. (A) IL-17 signaling pathway, (B) ribosome, (C) autoimmune thyroid disease, (D) systemic lupus erythematosus, (E) pertussis, and (F) antigen processing and presentation. IL-17 = interleukin-17, NDRG3 = N-Myc downstream-regulated gene 3.

3.2. Correlation analysis of NDRG3 methylation

To elucidate the biological significance of NDRG3 methylation in EOC, 183 genes associated with NDRG3 methylation were screened (FDR < .01).

Gene ontology enrichment analysis indicates (Fig. 5A–C and Table 2) that in biological process terms, co-expressed genes related to NDRG3 methylation are primarily engaged in mitochondrial respiratory chain complex assembly (GO:0033108), nicotinamide adenine dinucleotide hydride dehydrogenase complex assembly (GO:0010257), translational elongation (GO:0006414), mitochondrial gene expression (GO:0140053), and protein localization to the endoplasmic reticulum (GO:0070972). Regarding cellular components, the co-expressed genes are mainly involved in mitochondrial protein complex (GO:0098798), respiratory chain (GO:0070469), mitochondrial inner membrane (GO:0005743), nicotinamide adenine dinucleotide hydride dehydrogenase complex (GO:0030964), and ribosome (GO:0005840). In terms of molecular functions, these genes primarily participate in structural constituent of ribosome (GO:0003735), oxidoreductase activity acting on NAD(P)H (GO:0016651), rRNA binding (GO:0019843), electron transfer activity (GO:0009055), and phosphotransferase activity with phosphate group as acceptor (GO:0016776). Further KEGG pathway Enrichment Analysis demonstrates that pathways such as ribosome (hsa03010), Parkinson disease (hsa05012), oxidative phosphorylation (hsa00190), Alzheimer disease (hsa05010), and proteasome (hsa03050) are closely associated with NDRG3 methylation (Fig. 5D and Table 2).

3.3. NDRG3 protein–protein interaction network analysis

A possible NDRG3 PPI network map was constructed using the STRING database (Fig. 6). The results showed that the top 10 proteins that interacted with NDRG3 were TARDBP, CSTF1, NPEPL1, HDHD3, EDEM2, TRPC4AP, PHF20, SLA2, and SULF2.

3.4. High NDRG3 expression in EOC

NDRG3 mRNA expression in the 22 EOC patients was determined by qPCR. The results showed that the NDRG3 expression level in EOC tumor tissues and in paracancerous tissues were 5.15 ± 4.21 and 1.49 ± 1.38 times that of the internal control GAPDH ($P = .01$, Fig. 7A), respectively. The expression level in EOC tumor tissue was approximately 3.5 times higher than that in paracancerous tissues. IHC revealed that 71 of 110 EOC cases exhibited high NDRG3 protein expression, whereas only 14 paraneoplastic tissues exhibited high expression ($\chi^2 = 62.29$, $P < .001$, Fig. 7B). Therefore, NDRG3 can be used as a potential diagnostic indicator for EOC. Typical IHC-stained images of EOC tumors are shown in Figure 8, and according to the images, the positively stained areas were primarily localized in the cell membrane and cytoplasm. Our findings show that NDRG3 was highly expressed in the tumor tissues of EOC patients.

3.5. High NDRG3 expression is correlated with the clinicopathological features of EOC

Our statistical analysis showed that high NDRG3 expression was related to the clinicopathological features of EOC (Table 3). High NDRG3 expression was correlated with distant metastasis ($P = .02$), tumor–node–metastasis (TNM) stage ($P = .03$), and prognosis ($P = .01$) in EOC patients. Other major clinical indicators such as age, tumor size, histological type, pathological grade, and lymph node metastasis were not significantly related to NDRG3 expression.

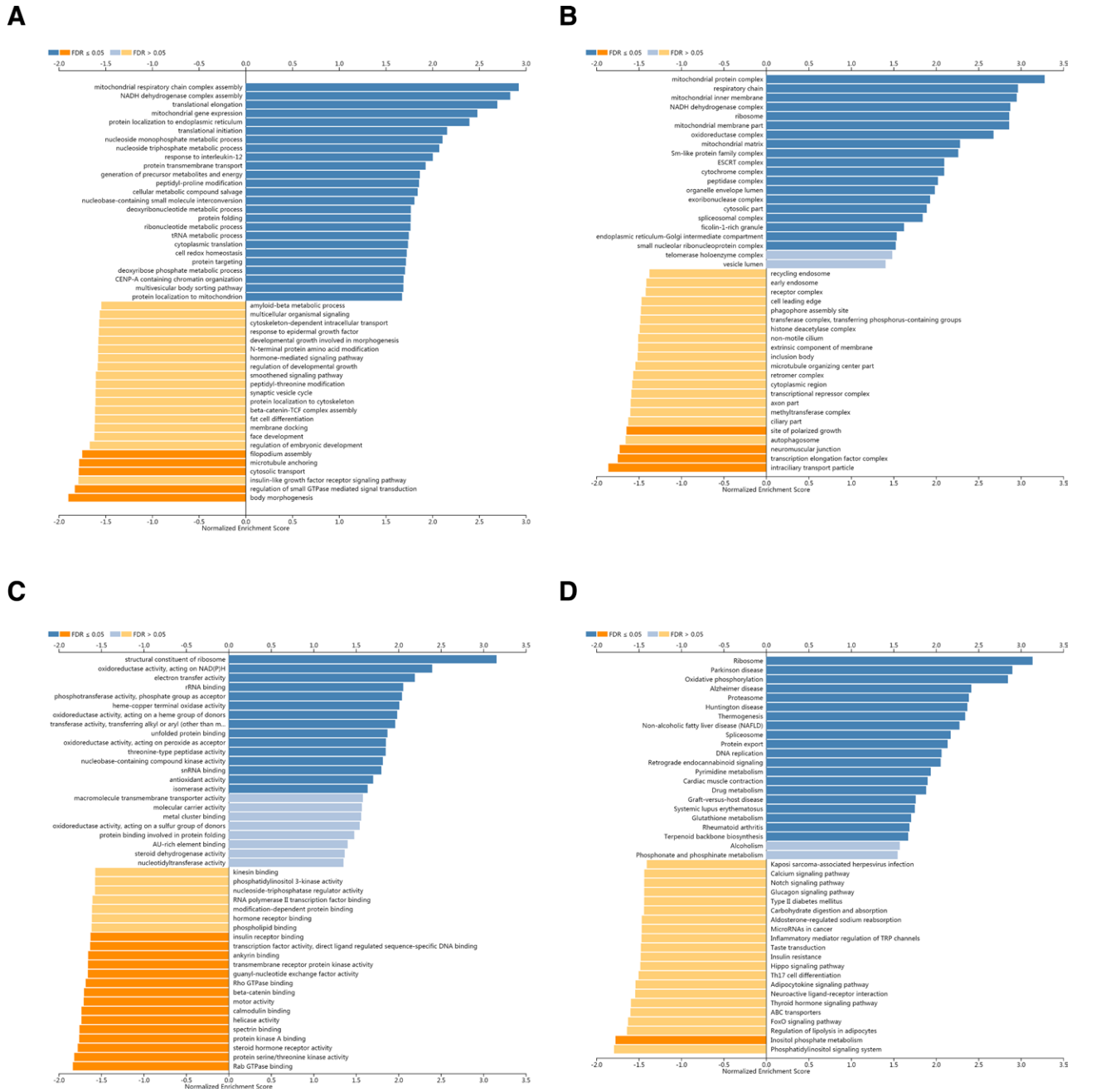

Figure 5. Gene ontology biological process enrichment analysis of NDRG3 methylation-related genes in EOC. (A) Biological processes, (B) cellular composition, (C) molecular functions, and (D) KEGG pathways. EOC = epithelial ovarian cancer, KEGG = Kyoto encyclopedia of genes and genomes, NDRG3 = N-Myc downstream-regulated gene 3.

3.6. High NDRG3 expression is correlated with the survival of EOC patients

We examined the Kaplan–Meier curve and found that high NDRG3 expression ($P = .001$), a high pathological grade ($P < .01$), lymph node metastasis ($P < .001$), distant metastasis ($P < .001$), and a high TNM stage ($P < .001$) were associated with short overall survival (OS) of EOC patients. In addition, high NDRG3 expression ($P < .001$) and lymph node metastasis ($P < .001$) were correlated with shortened disease-free survival (DFS) of EOC patients in this study (Fig. 9). The univariate analysis results in Table 4 show that high NDRG3 expression ($P < .001$), high pathological grade ($P < .05$), lymph node metastasis ($P < .001$), and TNM stage ($P = .02$) were associated with DFS in EOC patients in this study; similar statistical results in Table 5 suggest that high NDRG3 expression ($P < .01$), tumor pathological grade ($P < .01$), lymph node metastasis ($P < .001$), distant metastasis ($P < .001$), and TNM stage ($P < .001$) were associated with OS. In contrast, the multivariate analysis results in Tables 4 and 5 show that high NDRG3 expression was an independent prognostic factor for DFS and OS in EOC patients ($P = .01$, $P < .05$, respectively) and that lymph node metastasis was an independent prognostic indicator for both DFS and OS ($P = .01$, $P = .02$, respectively).

4. Discussion

The NDRG family includes the 4 homologs NDRG1, NDRG2, NDRG3, and NDRG4. Among them, NDRG3 has many

Table 2

Enriched GO and KEGG items.

Enriched category	Description	Count	NES	*P*-value	FDR
Biological process					
GO:0033108	Mitochondrial respiratory chain complex assembly	68	3.011	<.001	<0.001
GO:0010257	NADH dehydrogenase complex assembly	49	2.918	<.001	<0.001
GO:0006414	Translational elongation	123	2.789	<.001	<0.001
GO:0140053	Mitochondrial gene expression	142	2.481	<.001	<0.001
GO:0070972	Protein localization to endoplasmic reticulum	135	2.441	<.001	<0.001
Cellular components					
GO:0098798	Mitochondrial protein complex	213	3.280	<.001	<0.001
GO:0070469	Respiratory chain	84	2.965	<.001	<0.001
GO:0005743	Mitochondrial inner membrane	369	2.951	<.001	<0.001
GO:0030964	NADH dehydrogenase complex	43	2.875	<.001	<0.001
GO:0005840	Ribosome	216	2.862	<.001	<0.001
Molecular function					
GO:0003735	Structural constituent of ribosome	152	3.156	<.001	<0.001
GO:0016651	Oxidoreductase activity, acting on NAD(P)H	96	2.398	<.001	<0.001
GO:0019843	rRNA binding	58	2.055	<.001	0.001
GO:0009055	Electron transfer activity	105	2.193	<.001	0.001
GO:0016776	Phosphotransferase activity, phosphate group as acceptor	33	2.042	<.001	0.001
KEGG pathway					
hsa03010	Ribosome	129	3.137	<.001	<0.001
hsa05012	Parkinson disease	115	2.899	<.001	<0.001
hsa00190	Oxidative phosphorylation	104	2.847	<.001	<0.001
hsa05010	Alzheimer disease	152	2.416	<.001	<0.001
hsa03050	Proteasome	44	2.386	<.001	<0.001

FDR = false discovery rate, GO = gene ontology, KEGG = kyoto encyclopedia of genes and genomes, NADH = nicotinamide adenine dinucleotide hydride, NES = normalized enrichment score.

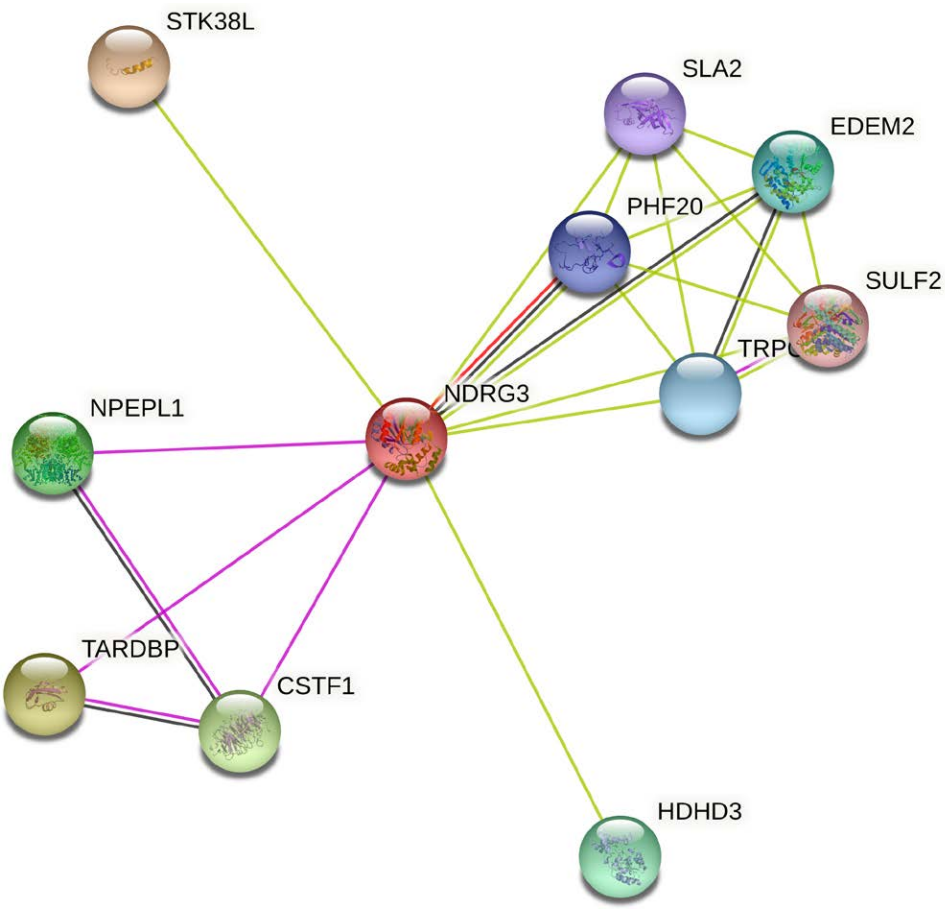

Figure 6. Protein–protein interaction network map.

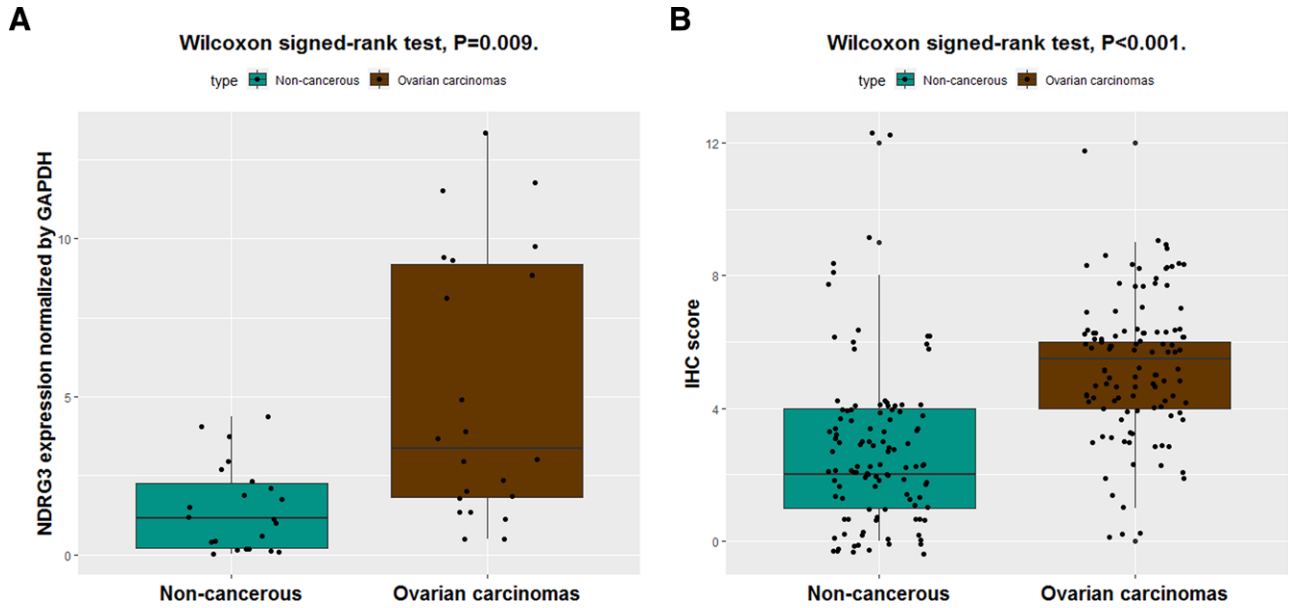

Figure 7. NDRG3 mRNA expression in EOC tissues and matched noncancerous tissues. (A) 1-step qPCR and IHC were performed to evaluate NDRG3 mRNA and protein expression in EOC tissues and matched noncancerous tissues. *NDRG3* mRNA expression in EOC tissues was significantly higher than that in corresponding tumor-adjacent noncancerous tissues ($P = .01$) when normalized to that of the internal control GAPDH. (B) NDRG3 protein expression in EOC tissues was higher than that in matched tumor-adjacent noncancerous tissues according to IHC. IHC scoring data showed that NDRG3 protein expression in EOC tissues was significantly higher than that in matched tumor-adjacent noncancerous tissues ($P < .001$). EOC = epithelial ovarian cancer, GAPDH = glyceraldehyde 3-phosphate dehydrogenase, IHC = immunohistochemistry, NDRG3 = N-Myc downstream-regulated gene 3, qPCR = quantitative polymerase chain reaction.

important physiological functions, including in embryonic development, cell differentiation, and cell proliferation.[13,14] Cui et al[25] found that NDRG3 was involved in the regulation of cell apoptosis. NDRG3 also has a specific relationship with hypoxia and ischemic stroke.[15–17] NDRG3 is associated with the invasion, metastasis, and prognosis of tumors. For example, in laryngeal cancer,[20] HCC,[19,21] lung cancer,[26] colorectal cancer,[27] gastric cancer,[18,28] breast cancer,[29] and others, high NDRG3 expression has been found to be associated with a poor prognosis. Du et al[30] found that a microRNA31–NDRG3 regulatory axis was essential for HCC survival and drug resistance. Shi et al[31] reported that high NDRG3 expression could regulate HCC metastasis by regulating β-catenin turnover. Thus, NDRG3 can serve as a potential target in targeted therapy for HCC, which warrants further in-depth study. Li et al[27] found that NDRG3, a tumor-promoting gene in colorectal cancer, could promote the migration and invasiveness of colorectal cancer cells by activating Src phosphorylation. NDRG3 might be an oncogene that can promote tumor growth and is associated with tumor prognosis. However, no studies of NDRG3 in EOC have been reported. Therefore, this study focuses on the clinicopathological associations between NDRG3 expression and EOC, particularly the relationship between high NDRG3 expression and the prognosis of EOC patients.

In this study, NDRG3 mRNA and protein expression levels in EOC tumor tissues were determined by qPCR and IHC, respectively. The findings suggested that NDRG3 was highly expressed in EOC tumor tissues. High NDRG3 expression was correlated with important clinicopathological features, such as TNM stage, lymph node metastasis, and prognosis. Statistical methods including univariate analysis, multivariate analysis, and Kaplan–Meier curves revealed not only that high NDRG3 expression correlated with the DFS and OS of EOC patients but that NDRG3 could also serve as an independent prognostic indicator in EOC. Therefore, NDRG3 can be considered an oncogene in EOC that is correlated with the clinicopathological features and prognosis of EOC.

In our PPI network analysis of NDRG3 in EOC, we identified 10 key proteins exhibiting the closest interaction with NDRG3: TARDBP, CSTF1, NPEPL1, HDHD3, EDEM2, TRPC4AP, PHF20, SLA2, and SULF2. This suggests the existence of potential mechanistic pathways and biological interactions between NDRG3 and these critical genes, interactions that may influence the progression of EOC. The interrelations, roles, and possible impacts of these proteins in conjunction with NDRG3 remain largely unexplored and warrant further investigation through a series of foundational experimental studies.

Finally, to further explore the regulatory network and signaling pathways of NDRG3, we performed a pearson correlation analysis of NDRG3 co-expressed genes using the LinkedOmics database. The analysis of differentially expressed genes associated with NDRG3 in EOC showed that NDRG3 exerted a broad impact on the transcriptome. In our study, the GO and pathway enrichment analyses revealed that NDRG3 is intricately involved in various biological processes and pathways. Key findings suggest that NDRG3 plays a significant role in the organ- or tissue-specific immune response, notably influencing the response to chemokines and interleukin-1 production. These processes are crucial for myeloid dendritic cell activation and influence responses to interleukin-12, highlighting NDRG3's potential impact on immune regulation. Additionally, NDRG3's association with the MHC protein complex and antigen binding suggests a role in antigen presentation. Structurally, NDRG3 is linked to transcriptionally active chromatin, autophagosome formation, and the microtubule organizing center, indicating its involvement in cellular architecture and intracellular transport. Functionally, its participation in oxidoreductase and antioxidant activities may contribute to cellular protection mechanisms. Pathway analysis identifies the IL-17 signaling pathway and systemic pathways, such as autoimmune thyroid disease and systemic lupus erythematosus, indicating broader implications in inflammatory and autoimmune conditions. These results align with the study's aim to elucidate NDRG3's multifaceted role in disease pathology, potentially offering new insights into therapeutic targets.

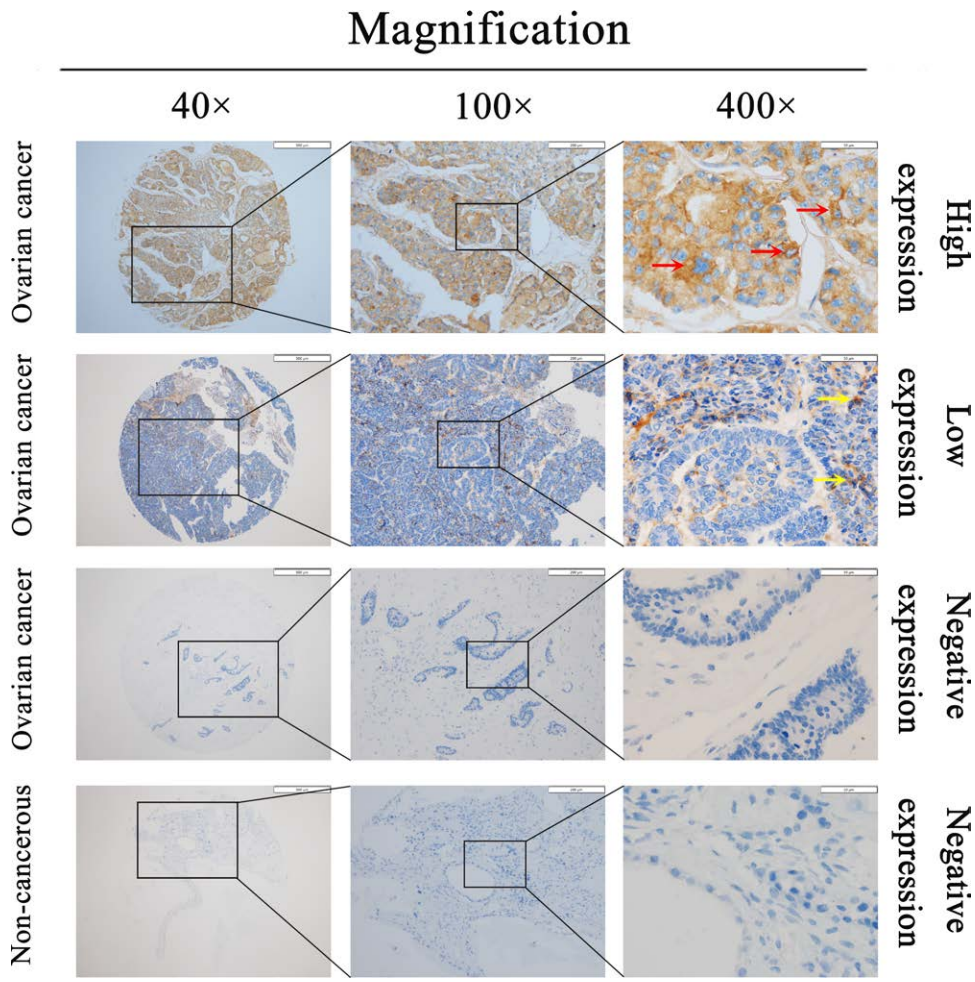

Figure 8. Representative images of NDRG3 protein expression in EOC tissues and corresponding noncancerous tissues. Representative images of NDRG3 protein expression in EOC tissues and corresponding noncancerous tissues. Positive staining was observed in the cytoplasm and membranes of cancer cells. EOC = epithelial ovarian cancer, NDRG3 = N-Myc downstream-regulated gene 3.

Some studies on NDRG3 have reported opposite conclusions. Estiar et al[32] reported that high NDRG3 expression could inhibit tumor proliferation in breast cancer and can therefore be exploited as a suppressor in breast cancer. Lee et al[33] found that NDRG3 expression could inhibit the metastatic capacity of prostate cancer cells. Consequently, we believe that further research on NDRG3 in tumors is needed. Many experimental factors can affect NDRG3 expression in different tumor types, such as limitations related to sample collection, histological type, and TNM stage, the source of the antibody used, and the quality control of the experiment.

The present study acknowledges several limitations that warrant further exploration. First, 1 significant limitation of our study is the relatively small sample size, comprising 110 EOC patients. This constraint may impact the generalisability of our results to a broader population. Future research should aim to replicate these findings in larger, more heterogeneous cohorts to strengthen the statistical power and credibility of our conclusions. Secondly, the data was gathered from a single institution, which might introduce selection bias. Conducting multicentre studies would enhance the robustness and applicability of our outcomes. Lastly, while our study offers valuable insights into the association of NDRG3 with EOC progression and prognosis, it falls short of elucidating the underlying molecular mechanisms in detail. Supplementary experimental investigations, including in vivo and in vitro studies, are essential to unravel the precise pathways through which NDRG3 affects tumor biology.

Future research should aim to dissect these specific pathways and investigate the possibility of NDRG3's involvement in other cancer-associated pathways. Given its prognostic potential, it becomes imperative to assess the viability of NDRG3 as a therapeutic target in subsequent studies. Investigating how NDRG3 interacts with other key proteins may unveil novel targets for drug development, potentially advancing therapeutic strategies. There is also a need for continuous research efforts to evaluate the applicability of NDRG3 as a biomarker for early detection and the development of personalized treatment regimens in EOC, which could significantly enhance patient management and clinical outcomes. Furthermore, long-term cohort studies that track patient outcomes in relation to NDRG3 expression levels are essential to provide a comprehensive understanding of its role and prognostic utility in a clinical setting.

5. Conclusions

In summary, in this study, the relationships between high NDRG3 expression and the clinicopathological characteristics of EOC were explored for the first time, especially the relationship between NDRG3 expression and prognosis. In this study, the role of NDRG3 methylation was studied in EOC, and proteins that possibly interact with NDRG3 were assessed using a bioinformatics analysis. We found that NDRG3 is a very valuable and promising tumor marker for EOC, which warrants further in-depth investigation. Studies with large sample sizes

Table 3

Relationship between NDRG3 overexpression and the clinicopathological characteristics of 110 cases of epithelial ovarian cancer.

Groups	No.	NDRG3		χ^2	*P*-value
		+	%		
Total	110	73	66.4		
Age (yr)					
≥60	35	20	57.1	1.69	.19
<60	76	53	69.7		
Tumor size (cm)					
>5	93	64	68.9	1.62	.20
≤5	17	9	52.9		
Histological type					
Serous	79	52	65.8	2.95	.40
Mucinous	13	11	84.6		
Endometroid	13	7	53.8		
Clear cell	5	3	60.0		
Pathological grade					
Grade 1	19	9	47.4	3.85	.15
Grade 2	18	12	66.7		
Grade 3	73	52	71.2		
Lymph node metastasis					
Positive	24	19	79.2	2.25	.13
Negative	86	54	62.8		
Distant metastasis					
Positive	27	23	85.2	5.68	.02*
Negative	83	50	60.2		
TNM stage					
Stage I	4	2	50.0	9.19	.03*
Stage II	12	5	41.7		
Stage III	65	41	63.1		
Stage IV	29	25	86.2		
Survival					
Yes	49	26	53.1	7.00	.01*
No	61	47	77.0		

NDRG3 = N-Myc downstream-regulated gene 3.

*$P < .05$.

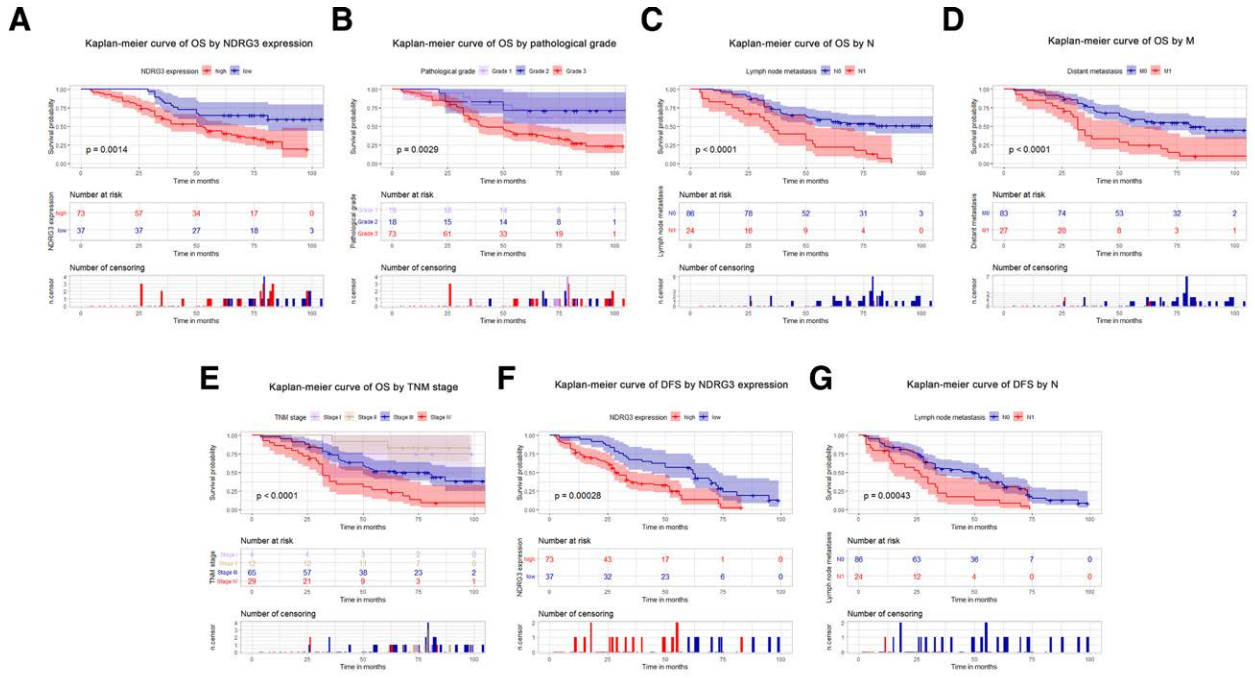

Figure 9. The prognostic value of NDRG3 expression for the OS and DFS of EOC patients. The Kaplan–Meier method was used to analyze the survival of EOC patients. Patients with high NDRG3 protein expression (A), advanced pathological grade (B), lymph node metastasis (N) (C), distant metastasis (M) (D), or advanced TNM stage (E) exhibited worse OS rates. Patients with high NDRG3 protein expression (F) or lymph node metastasis (N) (G) exhibited worse DFS rates. The *P*-value was determined using the log-rank test. DFS = disease-free survival, EOC = epithelial ovarian cancer, NDRG3 = N-Myc downstream-regulated gene 3, OS = overall survival, TNM = tumor node metastasis.

Table 4

Univariate and multivariate analyses on the disease-free survival of 110 cases of epithelial ovarian cancer.

	Univariate analysis			Multivariate analysis		
	HR	*P* > |z|	95% CI	HR	*P* > |z|	95% CI
NDRG3 expression High vs low	2.34	<.001*	1.451–3.783	2.02	.01*	1.224 to 3.348
Age (yr) ≥60 vs <60	1.16	.42	0.743 to 1.811			
Tumour size (cm) >5 vs ≤5	1.35	.32	0.747 to 2.447			
Histological type Serous vs mucinous vs endometroid vs clear cell	0.86	.28	0.658 to 1.129			
Pathological grade Grades 1 and 2 vs Grade 3	1.36	<.05*	1.003 to 1.829	1.18	.29	0.869 to 1.613
Lymph node metastasis Positive vs negative	2.32	<.001*	1.422 to 3.774	2.00	.01*	1.173 to 3.418
Distant metastasis Positive vs negative	1.48	.12	0.900 to 2.449			
TNM stage Stage I vs Stage II vs Stage III vs Stage IV	1.45	.02*	1.066 to 1.976	1.06	.73	0.758 to 1.484

CI = confidence interval, HR = hazard ratio, TNM = tumor node metastasis.
*$P < .05$.

Table 5

Univariate and multivariate analyses on the overall survival of 110 cases of epithelial ovarian cancer.

	Univariate analysis			Multivariate analysis		
	HR	*P* >|z|	95% CI	HR	*P* >|z|	95% CI
NDRG3 expression High vs low	2.55	<.05*	1.395 to 4.650	1.90	.04*	1.024 to 3.524
Age (yr) ≥60 vs <60	0.88	.65	0.512 to 1.516			
Tumour size (cm) >5 vs ≤5	1.42	.36	0.672 to 3.007			
Histological type Serous vs mucinous vs endometroid vs clear cell	0.77	.14	0.548 to 1.091			
Pathological grade Grades 1 and 2 vs Grade 3	1.80	<.01*	1.197 to 2.711	1.40	.10	0.934 to 2.101
Lymph node metastasis Positive vs negative	3.14	<.001*	1.853 to 5.311	2.00	.02*	1.113 to 3.586
Distant metastasis Positive vs negative	2.95	<.001*	1.734 to 5.004	0.73	.57	0.252 to 2.142
TNM stage Stage I vs Stage II vs Stage III vs Stage IV	2.59	<.001*	1.701 to 3.953	2.18	.08	0.901 to 5.272

NDRG3 = N-Myc downstream-regulated gene 3.
*$P < .05$.

and mechanistic experiments to determine the effect of NDRG3 expression on the biological behaviors of EOC cells in vitro and in vivo are currently underway.

Author contributions

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Software: Ling Sha, Xuefeng Gu.
Supervision: Xuefeng Gu.
Visualization: Ling Sha, Xuefeng Gu.
Writing – original draft: Chuanbing Shi.

References

[1] Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209–49.
[2] Pan R, Zhu M, Yu C, et al; China Kadoorie Biobank Collaborative Group. Cancer incidence and mortality: a cohort study in China, 2008-2013. Int J Cancer. 2017;141:1315–23.
[3] Coburn SB, Bray F, Sherman ME, Trabert B. International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. Int J Cancer. 2017;140:2451–60.
[4] Oronsky B, Ray CM, Spira AI, Trepel JB, Carter CA, Cottrill HM. A brief review of the management of platinum-resistant-platinum-refractory ovarian cancer. Med Oncol. 2017;34:103.
[5] Cortez AJ, Tudrej P, Kujawa KA, Lisowska KM. Advances in ovarian cancer therapy. Cancer Chemother Pharmacol. 2018;81:17–38.
[6] Lee JM, Minasian L, Kohn EC. New strategies in ovarian cancer treatment. Cancer. 2019;125(Suppl 24):4623–9.
[7] Di Lorenzo G, Ricci G, Severini GM, Romano F, Biffi S. Imaging and therapy of ovarian cancer: clinical application of nanoparticles and future perspectives. Theranostics. 2018;8:4279–94.

[8] Chandra A, Pius C, Nabeel M, et al. Ovarian cancer: current status and strategies for improving therapeutic outcomes. Cancer Med. 2019;8:7018–31.
[9] Tan L, Sha L, Hou N, Zhang M, Ma Q, Shi C. High alpha B-crystallin and p53 co-expression is associated with poor prognosis in ovarian cancer. Biosci Rep. 2019;39:BSR20182407.
[10] Shimono A, Okuda T, Kondoh H. N-myc-dependent repression of ndr1, a gene identified by direct subtraction of whole mouse embryo cDNAs between wild type and N-myc mutant. Mech Dev. 1999;83:39–52.
[11] Shaw E, McCue LA, Lawrence CE, Dordick JS. Identification of a novel class in the alpha/beta hydrolase fold superfamily: the N-myc differentiation-related proteins. Proteins. 2002;47:163–8.
[12] Kim KR, Kim KA, Park JS, et al. Structural and biophysical analyses of human N-Myc downstream-regulated Gene 3 (NDRG3) protein. Biomolecules. 2020;10:90.
[13] Melotte V, Qu X, Ongenaert M, et al. The N-myc downstream regulated gene (NDRG) family: diverse functions, multiple applications. FASEB J. 2010;24:4153–66.
[14] Yang Q, Zhang X, Shi Y, et al. Increased expression of NDRG3 in mouse uterus during embryo implantation and in mouse endometrial stromal cells during in vitro decidualization. Reprod Sci. 2018;25:1197–207.
[15] Park KC, Lee DC, Yeom YI. NDRG3-mediated lactate signaling in hypoxia. BMB Rep. 2015;48:301–2.
[16] Lee DC, Sohn HA, Park ZY, et al. A lactate-induced response to hypoxia. Cell. 2015;161:595–609.
[17] Yao Y, Wang W, Jing L, et al. Let-7f regulates the hypoxic response in cerebral ischemia by targeting NDRG3. Neurochem Res. 2017;42:446–54.
[18] Liu Y, Xia J, Zhou Y, Shao S. High expression of NDRG3 correlates with poor prognosis in gastric cancer patients. Rev Esp Enferm Dig. 2021;113:524–8.
[19] Yin X, Yu H, He XK, Yan SX. Prognostic and biological role of the N-Myc downstream-regulated gene family in hepatocellular carcinoma. World J Clin Cases. 2022;10:2072–86.
[20] Ma J, Liu S, Zhang W, et al. High expression of NDRG3 associates with positive lymph node metastasis and unfavourable overall survival in laryngeal squamous cell carcinoma. Pathology (Phila). 2016;48:691–6.
[21] Jing JS, Li H, Wang SC, Ma JM, Yu LQ, Zhou H. NDRG3 overexpression is associated with a poor prognosis in patients with hepatocellular carcinoma. Biosci Rep. 2018;38:BSR20180907.
[22] Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018;46:D956–63.
[23] Zhang M, Sha L, Hou N, Shi C, Tan L. High expression of sperm-associated antigen 5 correlates with poor survival in ovarian cancer. Biosci Rep. 2020;40:BSR20193087.
[24] Gu XF, Shi CB, Zhao W. Prognostic value of carbonic anhydrase XII (CA XII) overexpression in hepatocellular carcinoma. Int J Clin Exp Pathol. 2019;12:2173–83.
[25] Cui C, Lin H, Shi Y, Pan R. Hypoxic postconditioning attenuates apoptosis via inactivation of adenosine A(2a) receptor through NDRG3-Raf-ERK pathway. Biochem Biophys Res Commun. 2017;491:277–84.
[26] Luo X, Hou N, Chen X, et al. High expression of NDRG3 associates with unfavorable overall survival in non-small cell lung cancer. Cancer Biomark. 2018;21:461–9.
[27] Li T, Sun R, Lu M, Chang J, Meng X, Wu H. NDRG3 facilitates colorectal cancer metastasis through activating Src phosphorylation. Onco Targets Ther. 2018;11:2843–52.
[28] Yu J, Shen J, Qiao X, et al. SNHG20/miR-140-5p/NDRG3 axis contributes to 5-fluorouracil resistance in gastric cancer. Oncol Lett. 2019;18:1337–43.
[29] Kim MC, Park MH, Kang SH, Bae YK. NDRG3 protein expression is associated with aggressive biologic phenotype and unfavorable outcome in patients with invasive breast cancer. Int J Clin Exp Pathol. 2019;12:3886–93.
[30] Du Z, Niu S, Xu X, Xu Q. MicroRNA31-NDRG3 regulation axes are essential for hepatocellular carcinoma survival and drug resistance. Cancer Biomark. 2017;19:221–30.
[31] Shi J, Zheng H, Yuan L. High NDRG3 expression facilitates HCC metastasis by promoting nuclear translocation of beta-catenin. BMB Rep. 2019;52:451–6.
[32] Estiar MA, Zare AA, Esmaeili R, et al. Clinical significance of NDRG3 in patients with breast cancer. Future Oncol. 2017;13:961–9.
[33] Lee GY, Shin SH, Shin HW, Chun YS, Park JW. NDRG3 lowers the metastatic potential in prostate cancer as a feedback controller of hypoxia-inducible factors. Exp Mol Med. 2018;50:1–13.