NDRG2 inhibits glycolysis in liver cancer cells is accompanied by the regulation of SIRT1

Benge Xin

Department of Interventional Radiology, The Second Affiliated Hospital of Air Force Medical University, 569 Xinsi Road, Baqiao District, Xi'an 710038, China.

wei cao (cawe-001@163.com)

Department of Interventional Radiology, The Second Affiliated Hospital of Air Force Medical University, 569 Xinsi Road, Baqiao District, Xi'an 710038, China.

Research Article

Keywords:

Posted Date: April 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1520286/v1

Abstract

NDRG2 is a tumor suppressor, highly expressed in normal tissues but down-regulated in many cancers. This enzyme was involved in glycolysis by regulating glycolytic enzymes in ccRCC and colorectal cancer. However, the mechanism of NDRG2 regulating glycolysis is still not clear, and the function of NDRG2 in liver tumor glycolysis is completely unknown. In the present study, we first demonstrated the low expression of NDRG2 in liver tumor tissues and found that the survival rate of patients was negatively correlated with the expression of NDRG2. Subsequently, based on NDRG2 overexpressed and knockdown cell lines, NDRG2 was shown to have the ability to inhibit glycolysis in liver cancer cells. Finally, SIRT1, a deacetylase that plays important role in glycolysis regulation, is negatively regulated by NDRG2 in liver tumors. Our data enrich the understanding of the role of NDRG2 in tumor growth and improve the mechanism by which NDRG2 regulates glycolysis.

Introduction

Liver cancer ranks among the top three malignant tumors in terms of mortality due to its high heterogeneity, high mortality, strong invasiveness, difficult cure, and poor prognosis(1).

As we know, NDRG2 (N-Myc downstream-regulated gene 2) is a tumor suppressor and was observed that down-regulated or even lost in various types of cancer(2, 3). The overexpression of NDRG2 can inhibit the growth, and promote the apoptosis of cancer cells(4). Moreover, in the liver tumor, researchers have demonstrated that overexpression of NDRG2 can inhibit cell migration and invasion by down-regulating CD24 expression(5) and MMPs (matrix metalloproteinase) (6, 7). All these reports proved the correlation between NDRG2 and liver cancer metastasis.

The glycolysis is a hallmark of cancer cells after metabolic reprogramming(8, 9). Two studies reported the regulatory roles of NDRG2 on glycolysis in colorectal cancer cells(10, 11). The overexpression of NDRG2 in colorectal cancer cells reduced glucose consumption, lactate production, and increased oxygen consumption; knockdown of NDRG2 increased glucose consumption, lactate production increased, and decreased oxygen consumption, suggesting that NDRG2 has an inhibitory effect on glucose glycolysis metabolism of tumor cells. Studies show that NDRG2 reduces colorectal cancer cells proliferation maybe because NDRG2 can reduce glucose uptake by down-regulating glucose transport and metabolism-related enzymes such as HK2, PKM2, GLUT1, and LDHA, and up-regulating the expression of TNXIP. Wei Shi et al also demonstrated that NDRG2 inhibits glycolysis in ccRCC in the same way(12). However, whether SIRT1 plays the same role in liver cancer remains unclear.

SIRT1(Silent Mating Type Information Regulator 1, SIRT1) belongs to the Sirtuin family and is an NAD + dependent histone deacetylase (13–15). SIRT1-mediated deacetylation inhibits the functions of multiple tumor suppressors, including P53(16), P73(17), and HIC1(18), suggesting that SIRT1 plays a promotive role in tumorigenesis and progression. Emerging studies have shown that tumor progression affected by SIRT1 may be an important way of regulating glycolysis. SIRT1 stimulates the expression of glycolysis-related genes, such as GLUT1 and GAPDH, and thus promotes glycolysis in tumors(19–21). What's more, SIRT1 can also interact with GAPDH and retains it in the cytosol, thus protecting the enzyme from nuclear translocation, and promoting glycolysis(22). Based on the above reports, whether there is a possible correlation between NDRG2 and SIRT1 has aroused our attention.

In the present study, we focused on the roles of NDRG2 in regulating glycolysis and in regulating SIRT1 expression in liver cancer cells. Our data indicate a novel pathway of NDRG2 regulates glycolysis in liver tumors.

Materials And Methods

Patient information and tissue specimens

This study was approved by the Ethics Committee of the Air Force Medical University. Fresh liver cell carcinoma specimens were collected from 143 patients at the Tangdu Hospital of the Air Force Medical University (Xi'an, China) from 2004 to2008. Liver tumor tissues were obtained from resected tumors and confirmed by pathological review. Liver tumor specimens were staged following American Joint Cancer Committee/Union for International Cancer Control (AJCC/UICC) classification guidelines. The grading and histopathology subtyping of Liver tumor specimens was based on WHO criteria.

Immunohistochemistry

Immunohistochemical staining was performed to assess the protein expression of NDRG2 as described previously(23). For immunohistochemistry, formalin-fixed tumor tissues were embedded in paraffin, and serial 4 mm sections were obtained using a Leica microtome. For staining, tumor sections were dewaxed in toluene, rehydrated in an alcohol gradient, permeabilized in citrate buffer (pH 6.0), quenched with 3% H2O2 for 5 min to eliminate endogenous peroxidase activity, washed in PBS, incubated overnight with different antibodies and then with biotinylated goat anti-rat or anti-rabbit IgG antibody for15 min. After washing, sections were incubated with streptavidin peroxidase, lightly counterstained with hematoxylin, and observed under a photomicroscope.

Cell lines and cell culture

HepG2 and SMMC-7721 cells were purchased from Merck Millipore (USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen Life Technologies, Carlsbad, CA, USA) at 37°C in an incubator (5% CO_2, 21% O_2 and 74% N_2) atmosphere.

Lentivirus generation and infection

Recombinant lentiviral vectors were constructed with Invitrogen's ViraPower™ Lentiviral System in our laboratory. The cDNAs of human NDRG2 were cloned and subcloned into the vector pLenti6. Short hairpin RNAs (shRNA) against human NDRG2 were designed using a small interfering RNA design program and were then subcloned into the EcoR I/Age I sites of the pLKO-TRC vector. The shRNA sequences specific for NDRG2 were as follows:

shNDRG2-1 forward:

5'-CCGGGAGGACATGCAGGAAATCATTCTCGAGAATGATTTCCTGCATGTCCTCTTTTTG-3';

shNDRG2-1reverse:

5'-AATTCAAAAAGAGGACATGCAGGAAATCATTCTCGAGAATGATTTCCTGCATGTCCTC-3';

shNDRG2-2 forward:

5'-
CGGGATCCAAAAAAGCCACCTCAAGCGTCCGTCCTAGCAACAGCAAGCTTCCTGTTGCCAGGACAGACGCCTGAGGCGGCGGTGTTTCGTCCTTTCCACAA-3'

shNDRG2-2reverse:
5'- CCCTCGAGCCCCAGTGGAA- 3'

The sequences for the control nonsense shRNA were as follows:

controlforward:

5'-CCGGAAGGTCTTGTCCTCATCAACACTCGAGTGTTGATGAGGACAAGACCTTTTTTTG-3';

controlreverse:

5'–AATTCAAAAAAAGGTCTTGTCCTCATCAACACTCGAGTGTTGATGAGGACAAGACCTT-3'.

The HEK-293T cells were transfected with the pLenti6-Cherry/NDRG2, pLKO-Scramble/NDRG2-shRNA, PAX2, and PMD2G lentiviral vectors using Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). After 48 h, the lentiviral supernatants were collected, filtered (0.45 μm size filter; Millipore, Billerica, MA, USA), and added onto the HepG2/SMMC-7721 cells in the presence of 2 μg/ml Blasticidin (Sigma-Aldrich, USA) or 1 μg/ml Polybrene (Sigma-Aldrich, USA) for 6 to 8 h. Two rounds of infection were performed. After infection, the cells that survived this treatment were selected for a week, and then being analyzed for NDRG2 expression by Western blot.

Western blot analysis

The cells were harvested from 60 mm culture dishes. Lysates of the collected cells were prepared by lysis in 200 ml RIPA buffer (0.05 M Tris- HCl [pH 7.4], 0.15 M NaCl, 0.25% deoxycholic acid, 1% Nonidet P-40, 1 mM EDTA, 1 mMphenylmethylsulfonyl fluoride, 10 mg/ml aprotinin and 10 mg/ml leupeptin), supplemented with 100:1 (v/v) ratios of a protease inhibitor cocktail and phosphatase inhibitor cocktails. The protein concentrations were measured using the bicinchoninic acid (BCA) protein assay. The proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were saturated with Tris-buffered saline with 0.1% Tween 20 and 3% bovine serum albumin (TBST-BSA) and were then probed with the appropriate antibodies: NDRG2 (1:2000, Cell Signaling Technology, Danvers, MA, USA), β-actin (1:2000, Cell Signaling Technology, USA), SIRT1 (1:1000 Cell Signaling Technology, USA), followed by incubation with species-matched secondary antibodies. The bands were detected using enhanced chemiluminescence (Pierce Rockford, IL, USA) or the Odyssey Imaging System (LiCor Biosciences). The band intensities were quantified with Kodak Digital Science 1D 3.0 (Eastman Kodak, New Haven, CT).

Measurement ofglucoseuptake, lactate production, LDH activity and oxygen consumption rate

Cells were seeded on 6-well plates at a density of 2 × 105 cells per well and incubated at 37°Cfor 24h. The concentrations of glucose and lactate in the culture medium were measured by Glucose TestKit (Invitrogen) and Lactate Assay Kit (JianchengBioengineering, Nanjing, China) individually. Harvested cells were digested with 0.25% trypsin and washed with PBS. The cell suspension was homogenized on ice. LDH activities were

measured by colorimetric assay using a specific test kit (Solarbio, Beijing, China) according to the manufacturer's suggestions. The absorbance of LDH was measured at 450 nm. The activity of LDH in the control group was normalized to 1.0.

The cellular activity and Oxygen Consumption Rate (OCR) were determined using the Seahorse XFe 96 Extracellular Flux Analyzer (Seahorse Bioscience). Experiments were performed according to the manufacturer's suggestion. OCR was examined using Seahorse XF Cell Mito Stress Test Kit (Seahorse Bioscience). Briefly, 2×105 cells were plated into Seahorse plates, maintained overnight, and then washed with Seahorse buffer. Next, Seahorse buffer including oligomycin (Oligo), p-trifluoromethoxy carbonyl cyanide phenylhydrazone (FCCP), and rotenone + antimycin A (Rot + AA) were sequentially injected. The results were analyzed using software XF-96 wave (Seahorse Bioscience). All experiments were repeated at least 3 times.

Statistical analysis

The chi-square test or Fisher's exact test and Student's t-test were utilized to determine the significance of the differences between groups. The survival rates were analyzed using Kaplan-Meier analysis and the log-rank test. The t-test method was used to compare the differences between the two groups, the analysis of variance method was used to compare the numbers of fibrosis nodules among groups. Statistical analysis was performed using SPSS software, Version 16.0 (Chicago, USA). The statistical significance was based on a value of $P < 0.05$.

Results

NDRG2 expression is down-regulated inlivertumor

NDRG2 was reported as a tumor suppressor, its expression was reduced in many tumor cells. Here, we first analyzed the information on NDRG2 mRNA levels in patients using Gene Expression Omnibus (GEO), a database repository of gene expression profiles. As shown in Fig. 1A-D, the mRNA level of NDRG2 ($P < 0.05$) was significantly decreased in liver tumors compared with peritumor from four sub-database (GSE14520, GSE22058, GSE25097, GSE36376). The protein level of NDRG2 was assessed in 143 liver carcinoma tissues and corresponding non-carcinoma tissues. The results of immunohistochemical staining showed that distinct staining can be detected in the cytoplasm of non-carcinoma tissues, but no staining or feeble staining can be detected in the liver tumor tissues(Fig. 1E). Such we conclude NDRG2 as a tumor suppressor is down-regulated in both mRNA and protein levels in liver tumors.

Correlation between NDRG2 expression and prognostic of liver cancer patients

We then investigated the correlation between NDRG2 expression and survival status of liver cancer patients using Kaplan-Meier curves. Figure 2 shows that patients with higher NDRG2 expression exhibited well overall survival (OS) ($p < 0.001$), Disease-specific survival (DSS) ($p < 0.001$), and relapse-free survival (RFS) ($p < 0.01$) compared with those with lower NDRG2 expression level. In detail, the mean OS of patients with high and low NDRG2 protein expression levels was 81.9 and 38.3 months, respectively. The mean DSS of patients with high and low NDRG2 protein expression levels was 84.7and 56.5 months, respectively. The mean RFS of patients with high and low NDRG2 protein expression levels was 37.2 and 15.17 months, respectively.

NDRG2 inhibits glycolysis in liver cancer cells

Glycolysis is a major feature of tumor cell metastasis (8, 9). To prove whether NDRG2 can also inhibit glycolysis in liver cancer cells as in colorectal cancer cells(10), we constructed two liver cancer cell lines with stable knockdown and overexpression of NDRG2 by using NDRG2-shRNA plasmid and NDRG2 eukaryotic expression plasmid, respectively (Fig. 3A and 3B). According to our data, overexpression of NDRG2 in liver cancer cell lines (SMMC-7721) inhibited glycolysis as suggested by decreased glucose uptake rate, decreased lactate production, decreased LDH activity, and increased oxygen consumption rate (OCR) (Figs. 3C-F).

Furthermore, NDRG2 knockdown by shRNA in liver cancer cells (HepG2) promoted glycolysis as indicated by increased glucose uptake rate, increased lactate production, increased LDH activity, and decreased oxygen consumption rate (OCR) (Fig. 3G). Taken together, these data demonstrated that NDRG2 inhibited glycolysis in liver cancer cells.

SIRT1 was down-regulated by NDRG2

To further explore the mechanism of NDRG2's involvement in glucose metabolism, the protein interaction network of NDRG2 was achieved using the STRING database (https://string-db.org/) (Fig. 4A). Impressively, SIRT1, a histone deacetylase that has been shown to play an important regulatory role in glucose metastasis (24), was found in this network despite without known or predicted interactions with NDRG2. Hence, we conceived that there was a correlation between NDRG2 and SIRT1 that may contribute to glucose metastasis in liver cancer cells. Subsequent western blot analysis proved that SIRT1 expression was increased in NDRG2 knockdown cells but was decreased in NDRG2 overexpressed cells (Figs. 4B and 4C). Our experimental data suggested the expression of SIRT1 was negatively correlated with that of NDRG2.

Discussion

NDRG2 is already known as a tumor suppressor, is involved in energy metabolism, especially glycosemetabolism(25, 26). In this study, the mRNA and protein expression levels of NDRG2 were analyzed in liver carcinoma tissues or non-carcinoma tissues, the results reconfirmed its down-expression in liver tumors (Fig. 1).

Recently, NDRG2 was involved in cellular glucose metabolism through insulin signal transduction based on the reports that NDRG2 is a substrate of kinase Akt and SGK1 (serum- and glucocorticoid-induced kinase 1)(27, 28). According to the Warburg effect, cancer cells are more prone to glycolysis than oxidative phosphorylation for glucose metabolism(29, 30). Glycolysis contributes to cancer progression(29), and glycolysis inhibition is emerging as a promising area of cancer therapy(31). Up to now, including in our study, NDRG2 has been shown to significantly inhibit glycolysis of tumor cells in colorectal cancer(10, 11), ccRCC(12), and liver cancer (Fig. 3C-F). In the light of these reports, the suppression of glycolysis by NDRG2 is the result of the regulation of glycolysis-related genes. Detailly, NDRG2 was firstly identified in breast cancer to decrease glucose uptake via promoting GLUT1 protein degradation without affecting GLUT1 transcription(32). Subsequently, the expression of glycolysis-related hexokinase 2 (HK2), pyruvate kinase M2 isoform (PKM2), and lactate dehydrogenase A (LDHA) were proved to be significantly suppressed by NDRG2 in colorectal cancer cells and ccRCC cells (10, 12). Moreover, NDRG2 could stimulate the TXINP expression to reduce glucose uptake(11).

Interestingly, although NDRG2 is named as an N-Mycdownstream-regulated gene it is not repressed by transcription factor N-Myc but by C-Myc(10, 33). C-Myc is known as a viral oncogene in cancer energy metabolism(34, 35) and mainly promotes glycolysis of cancer cells through up-regulating glycolysis genes expressions, such as LDH, HK2, GLUT1, and PKM2 (3). In addition, HIF-1 and P53, the two other transcription factors, also play crucial parts in tumorigenesis, could regulate the expression of glycolytic genes(36). HIF-1 promotes but P53 hinders these genes' expression(37, 38). Moreover, HIF-1 and P53 show negative and positive regulatory effects on NDRG2, respectively(39–42). Hence, we believe that the regulation of NDRG2 on glycolysis flux is accomplished by cooperating with C-Myc, HIF-1, and P53 to regulate the expression of glycolytic genes.

Sirtuins(SIRT1-7) play important roles in the Warburg effect and can regulate the glycolytic genes through their various effects(21). Sirtuins could directly regulate the expression of glycolytic enzymes, alter the enzymatic activity of glycolytic genes via multiple post-translational modifications and affect the sub-location of these enzymes(19, 21, 43, 44). For example, SIRT1was shown to promote the expression of GLUT1, GAPDH, and LDHA to benefit glycolysis(19, 43), interact with GAPDH, and keep it in the cytosol and thus promoting glycolysis expression(22). NDRG2 and SIRT1 showed opposite regulatory effects on the glycolytic enzyme, and this result may be due to the negative regulation of SIRT1 by NDRG2 (Fig. 4B-4C). The regulatory effects between SIRT1 and glycolytic regulators are opposite to that between NDRG2 and glycolytic regulators. The expression of SIRT1 increases through the C-Myc binding to the SIRT1 promoter, and then deacetylate C-Myc, and then stimulate the transcriptional activity of C-Myc(21); SIRT1-mediated deacetylation suppresses the functions of P53 (45). Overall, ndrg2 and sirt1, as a pair of negative regulatory genes, are opposite in the regulation of tumor glycolysis.

Declarations

Ethics approval and consent to participate (kindly mention the name of the Ethics Committee and the Ethical Approval Number)

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

Not applicable

Funding

Not applicable

Authors' contributions

Benge Xin: Methodology, Software, Data curation, Writing- Original draft preparation. **Wei Cao**: Conceptualization, Visualization, Investigation. Supervision. Validation. Writing- Reviewing and Editing.

Acknowledgements

Not applicable

References

1. Chen Z, Xie H, Hu M, et al. Recent progress in treatment of hepatocellular carcinoma. Am J Cancer Res 2020;10:2993–3036.
2. Deng Y, Yao L, Chau L, et al. N-Myc downstream-regulated gene 2 (NDRG2) inhibits glioblastoma cell proliferation. Int J Cancer 2003;106:342–7.
3. Hu W, Fan C, Jiang P, et al. Emerging role of N-myc downstream-regulated gene 2 (NDRG2) in cancer. Oncotarget 2016;7:209–23.
4. Cao W, Zhang JL, Feng DY, et al. The effect of adenovirus-conjugated NDRG2 on p53-mediated apoptosis of hepatocarcinoma cells through attenuation of nucleotide excision repair capacity. Biomaterials 2014;35:993–1003.
5. Zheng J, Li Y, Yang J, et al. NDRG2 inhibits hepatocellular carcinoma adhesion, migration and invasion by regulating CD24 expression. BMC Cancer 2011;11:251:1–9.
6. Lee DC, Kang YK, Kim WH, et al. Functional and clinical evidence for NDRG2 as a candidate suppressor of liver cancer metastasis. Cancer Res 2008;68:4210–20.
7. Guo Y, Ma J, Wu L, et al. Hyperthermia-induced NDRG2 upregulation inhibits the invasion of human hepatocellular carcinoma via suppressing ERK1/2 signaling pathway. PLoS One 2013;8:e61079.
8. DeBerardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv 2016;2:e1600200.
9. Luengo A, Gui DY, Vander Heiden MG. Targeting Metabolism for Cancer Therapy. Cell Chem Biol 2017;24:1161–80.
10. Xu X, Li J, Sun X, et al. Tumor suppressor NDRG2 inhibits glycolysis and glutaminolysis in colorectal cancer cells by repressing c-Myc expression. Oncotarget 2015;6:26161–76.
11. Hu J, Feng L, Ren M, et al. Colorectal Cancer Cell Differentiation Is Dependent on the Repression of Aerobic Glycolysis by NDRG2-TXNIP Axis. Dig Dis Sci 2021.
12. Shi W, Xu X, Yan F, et al. N-Myc downstream-regulated gene 2 restrains glycolysis and glutaminolysis in clear cell renal cell carcinoma. Oncol Lett 2017;14:6881–7.
13. Kleszcz R, Paluszczak J, Baer-Dubowska W. Targeting aberrant cancer metabolism - The role of sirtuins. Pharmacol Rep 2015;67:1068–80.
14. Giblin W, Skinner ME, Lombard DB. Sirtuins: guardians of mammalian healthspan. Trends Genet 2014;30:271–86.
15. Mei Z, Zhang X, Yi J, et al. Sirtuins in metabolism, DNA repair and cancer. J Exp Clin Cancer Res 2016;35:182.
16. Lamichane S, Baek SH, Kim YJ, et al. MHY2233 Attenuates Replicative Cellular Senescence in Human Endothelial Progenitor Cells via SIRT1 Signaling. Oxid Med Cell Longev 2019;2019:6492029.
17. Dai JM, Wang ZY, Sun DC, et al. SIRT1 interacts with p73 and suppresses p73-dependent transcriptional activity. J Cell Physiol 2007;210:161–6.
18. Hallows WC, Yu W, Denu JM. Regulation of glycolytic enzyme phosphoglycerate mutase-1 by Sirt1 protein-mediated deacetylation. J Biol Chem 2012;287:3850–8.
19. Pinho AV, Mawson A, Gill A, et al. Sirtuin 1 stimulates the proliferation and the expression of glycolysis genes in pancreatic neoplastic lesions. Oncotarget 2016;7:74768–78.
20. Chen J, Cao L, Li Z, et al. SIRT1 promotes GLUT1 expression and bladder cancer progression via regulation of glucose uptake. Hum Cell 2019;32:193–201.
21. Zhu S, Dong Z, Ke X, et al. The roles of sirtuins family in cell metabolism during tumor development. Semin Cancer Biol 2019;57:59–71.
22. Joo HY, Woo SR, Shen YN, et al. SIRT1 interacts with and protects glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from nuclear translocation: implications for cell survival after irradiation. Biochem Biophys Res Commun 2012;424:681–6.
23. Cai BL, Li Y, Shen LL, et al. Nuclear Multidrug Resistance-Related Protein 1 Is Highly Associated with Better Prognosis of Human Mucoepidermoid Carcinoma through the Suppression of Cell Proliferation, Migration and Invasion. PLoS One 2016;11:e0148223.
24. Haigis MC, Guarente LP. Mammalian sirtuins–emerging roles in physiology, aging, and calorie restriction. Genes Dev 2006;20:2913–21.
25. Chen XL, Lei L, Hong LL, et al. Potential role of NDRG2 in reprogramming cancer metabolism and epithelial-to-mesenchymal transition. Histol Histopathol 2018;33:655–63.
26. Kim G, Lim S, Kim KD. N-myc Downstream-Regulated Gene 2 (NDRG2) Function as a Positive Regulator of Apoptosis: A New Insight into NDRG2 as a Tumor Suppressor. Cells 2021;10.
27. Burchfield JG, Lennard AJ, Narasimhan S, et al. Akt mediates insulin-stimulated phosphorylation of Ndrg2: evidence for cross-talk with protein kinase C theta. J Biol Chem 2004;279:18623–32.
28. Murray JT, Campbell DG, Morrice N, et al. Exploitation of KESTREL to identify NDRG family members as physiological substrates for SGK1 and GSK3. Biochem J 2004;384:477–88.
29. Vaupel P, Schmidberger H, Mayer A. The Warburg effect: essential part of metabolic reprogramming and central contributor to cancer progression. Int J Radiat Biol 2019;95:912–9.

30. Chen X, Xie C, Fan XX, et al. Novel direct AMPK activator suppresses non-small cell lung cancer through inhibition of lipid metabolism. Oncotarget 2017;8:96089–102.
31. Sheng H, Tang W. Glycolysis Inhibitors for Anticancer Therapy: A Review of Recent Patents. Recent Pat Anticancer Drug Discov 2016;11:297–308.
32. Ma J, Liu W, Guo H, et al. N-myc downstream-regulated gene 2 expression is associated with glucose transport and correlated with prognosis in breast carcinoma. Breast Cancer Res 2014;16:R27.
33. Zhang J, Li F, Liu X, et al. The repression of human differentiation-related gene NDRG2 expression by Myc via Miz-1-dependent interaction with the NDRG2 core promoter. J Biol Chem 2006;281:39159–68.
34. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res 2009;15:6479–83.
35. Mendez-Lucas A, Li X, Hu J, et al. Glucose Catabolism in Liver Tumors Induced by c-MYC Can Be Sustained by Various PKM1/PKM2 Ratios and Pyruvate Kinase Activities. Cancer Res 2017;77:4355–64.
36. Wu Z, Wu J, Zhao Q, et al. Emerging roles of aerobic glycolysis in breast cancer. Clin Transl Oncol 2020;22:631–46.
37. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
38. Li L, Liang Y, Kang L, et al. Transcriptional Regulation of the Warburg Effect in Cancer by SIX1. Cancer Cell 2018;33:368–85 e7.
39. Wang L, Liu N, Yao L, et al. NDRG2 is a new HIF-1 target gene necessary for hypoxia-induced apoptosis in A549 cells. Cell Physiol Biochem 2008;21:239–50.
40. Wang RX, Ou XW, Kang MF, et al. Association of HIF-1alpha and NDRG2 Expression with EMT in Gastric Cancer Tissues. Open Life Sci 2019;14:217–23.
41. Liu N, Wang L, Li X, et al. N-Myc downstream-regulated gene 2 is involved in p53-mediated apoptosis. Nucleic Acids Res 2008;36:5335–49.
42. Zhang K, Zhang Y, Zhang C, et al. Upregulation of P53 promotes nucleus pulposus cell apoptosis in intervertebral disc degeneration through upregulating NDRG2. Cell Biol Int 2021;45:1966–75.
43. Mao B, Zhao G, Lv X, et al. Sirt1 deacetylates c-Myc and promotes c-Myc/Max association. Int J Biochem Cell Biol 2011;43:1573–81.
44. Vettraino M, Manerba M, Govoni M, et al. Galloflavin suppresses lactate dehydrogenase activity and causes MYC downregulation in Burkitt lymphoma cells through NAD/NADH-dependent inhibition of sirtuin-1. Anticancer Drugs 2013;24:862–70.
45. Lin Z, Fang D. The Roles of SIRT1 in Cancer. Genes Cancer 2013;4:97–104.

Figures

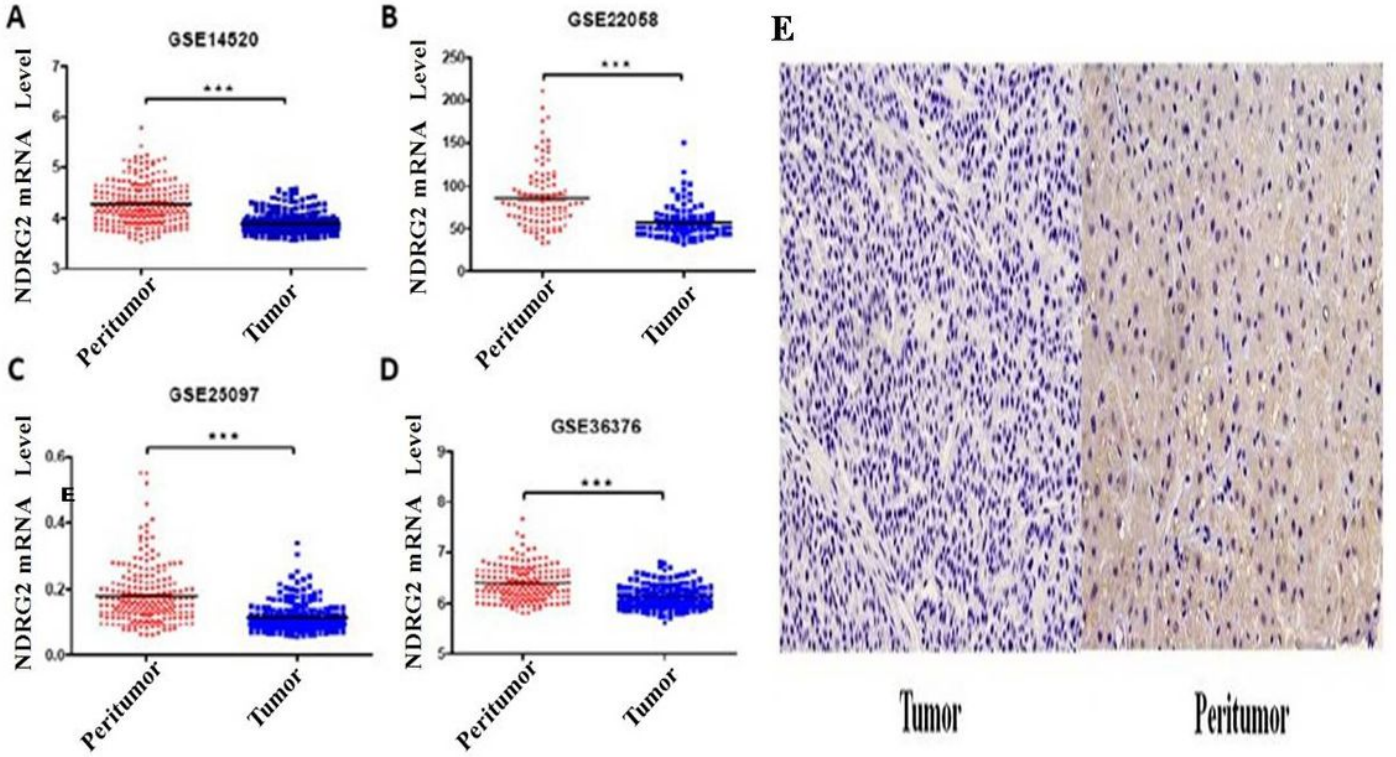

Figure 1

NDRG2 expression level was down-regulated in liver tumor. (A-D) NDRG2 mRNA expression level in liver tumor and peritumor analysis by four sub-database of Gene Expression Omnibus. (E) IHC assays was performed to detected the protein expression level of NDRG2 in human liver tumor tissues.

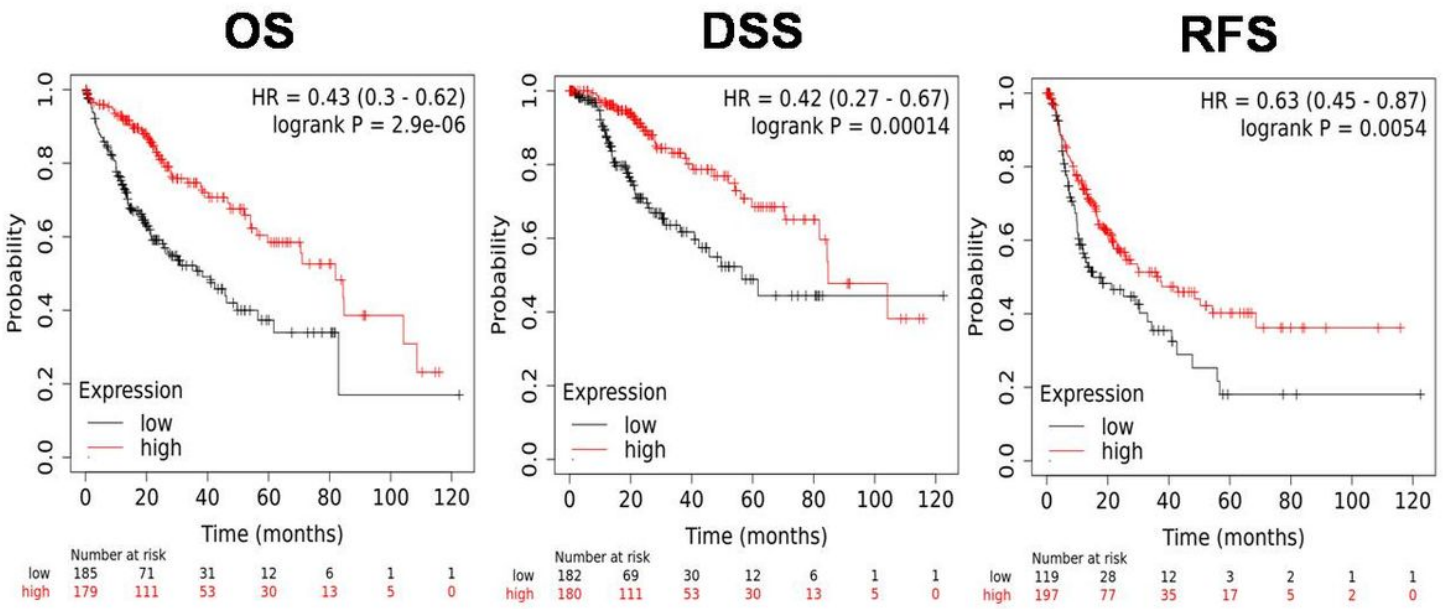

Figure 2

Kaplan-Meier curves of liver cancer patients survival rate with NDRG2 expression pattern. Results were acquired from online database Kaplan-Meier Plotter (kmplot.com). OS: overall survival; DSS: disease-specific survival; RFS: relapse free survival.

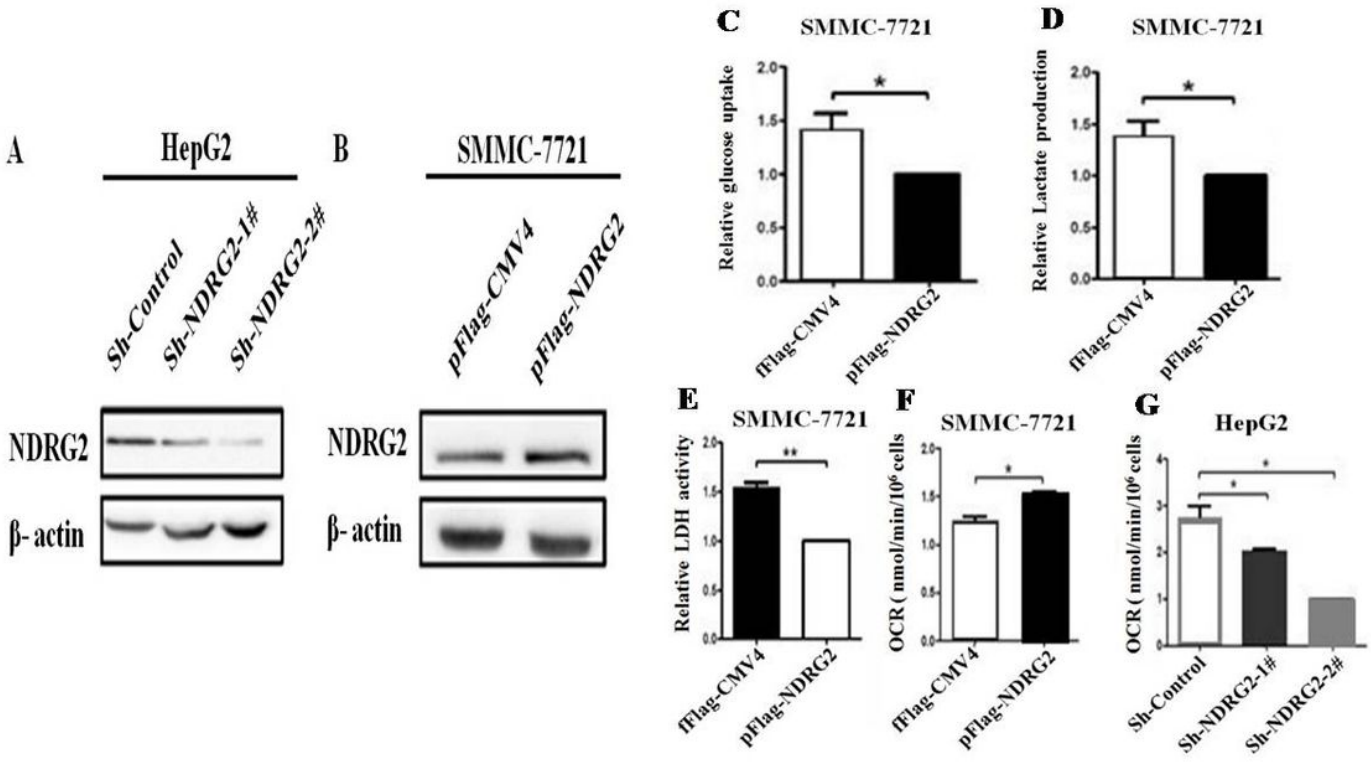

Figure 3

NDRG2 inhibits glycolysis in liver cancer cells. (A) NDRG2 down-regulation via viral delivery of shRNA in HepG2 cells. (B) NDRG2 up-regulation via viral delivery of pFlag-NDRG2 in SMMC-7721 cells. (C-F) NDRG2 overexpression inhibits glycolysis in SMMC-7721 cells as indicated by glucose uptake (C), lactate production (D), LDH activity (E), and oxygen consumption rate (F). (G) NDRG2 down-regulation hampers oxygen consumption rate in HepG2 cells.

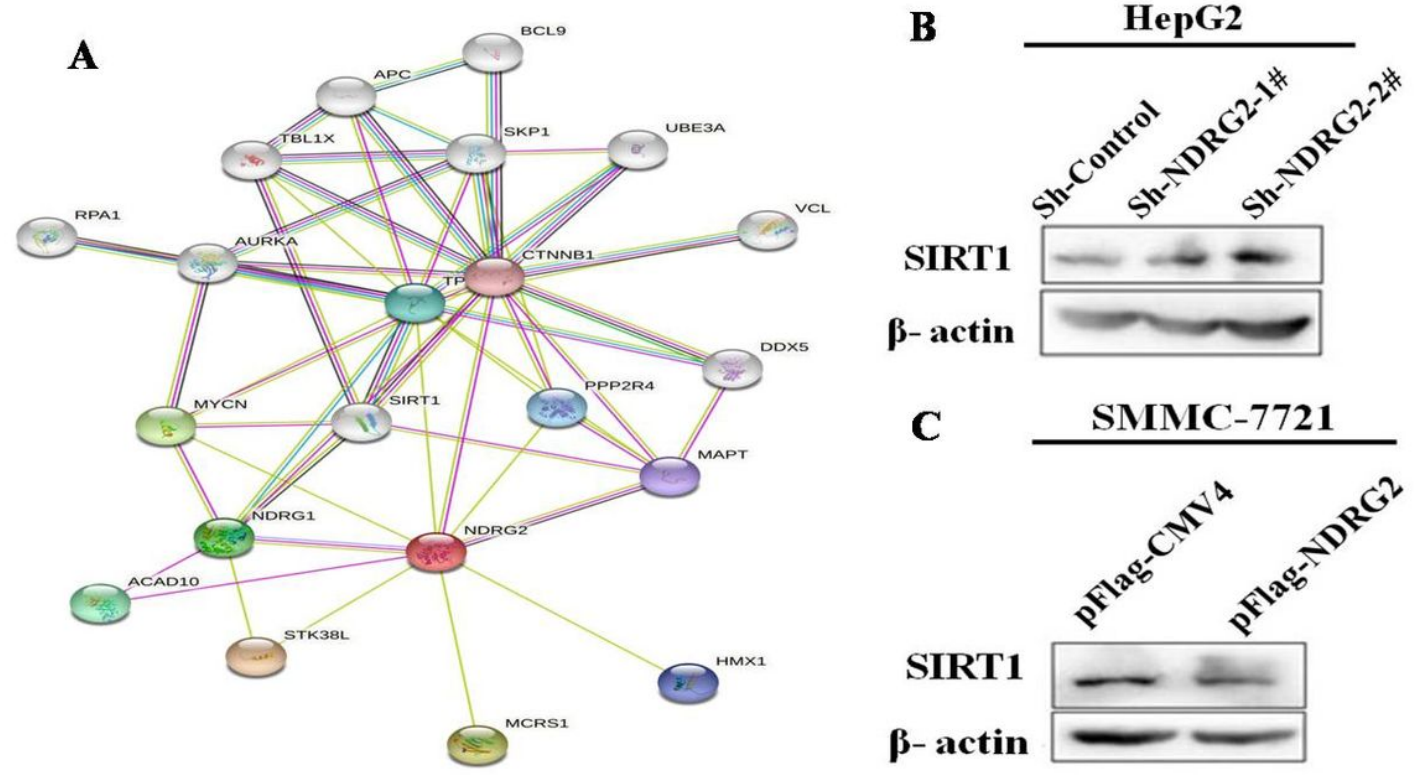

Figure 4

NDRG2 negatively regulate SIRT1 expression. (A and B) Western blot analysis of SIRT1 expression in NDRG2 down- (A) and up-regulated cells. (C) Analysis of NDRG2's protein interaction network using the online STRING database.