Chapter

N-Myc Downstream-Regulated Gene 2 (NDRG2) as a Novel Tumor Suppressor in Multiple Human Cancers

Jian Zhang, Xia Li, Liangliang Shen, Yan Li and Libo Yao

Abstract

N-myc downstream-regulated gene 2 (NDRG2) was identified as a novel tumor suppressor gene in regulating the proliferation, differentiation, apoptosis and metastasis of multiple cancer types. Consistent with this finding, we and other groups observed the decreased NDRG2 expression in multiple human cancer cell lines and tumors, including breast cancer, colorectal cancer, and cervical cancer. We identified NDRG2 as a stress sensor for hypoxia, DNA damage stimuli and endoplasmic reticulum stress (ERS). Our recent data showed that NDRG2 could promote the differentiation of colorectal cancer cells. Interestingly, we found that reduced NDRG2 expression was a powerful and independent predictor of poor prognosis of colorectal cancer patients. Furthermore, NDRG2 can inhibit epithelial-mesenchymal transition (EMT) by positively regulating E-cadherin expression. Moreover, NDRG2-deficient mice show spontaneous development of various tumor types, including T-cell lymphomas, providing in vivo evidence that NDRG2 functions as a tumor suppressor gene. We believe that NDRG2 is a novel tumor suppressor and might be a therapeutic target for cancer treatment.

Keywords: NDRG2, tumor suppressor, stress sensor, p53, differentiation, EMT, metastasis, cancer metabolism

1. The finding of NDRG2

The human NDRG2 sequence was first described by Deng et al. as a protein containing an acyl-carrier protein (ACP)-like domain [1, 2]. The gene was cloned from differentially expressed genes between glioblastoma and normal brain tissues using PCR-based subtractive hybridization in 2003 [2]. NDRG2, NDRG1, NDRG3, and NDRG4 comprise the NDRG gene family and share approximately 59–68% homology. Additionally, NDRG family members display over 92% homology between humans and mice [1].

We identified NDRG2 as a novel tumor suppressor gene that plays a role in regulating the proliferation, differentiation, apoptosis and metastasis of multiple types of malignant tumors [1, 2]. Consistent with this finding, NDRG2 downregulation has been observed in multiple human cancer cell lines and tumors [3–5]. Additionally, other groups later confirmed our finding [6, 7]. NDRG2 was identified as a stress sensor for hypoxia, DNA damage stimuli and endoplasmic reticulum stress (ERS), and could inhibit the proliferation and promote the differentiation of colorectal

carcinoma cells [8]. Moreover, NDRG2-deficient mice show spontaneous development of various tumor types, providing in vivo evidence that NDRG2 functions as a tumor suppressor gene. In this chapter, we will introduce the recent findings of NDRG2 as tumor suppressor in vitro and in vivo, and also the detailed mechanism.

2. NDRG2 as a hypoxia and DNA damage responder

Our group firstly identified NDRG2 as a protein containing an acyl-carrier protein (ACP)-like domain. The gene was cloned from differentially expressed genes between glioblastoma and normal brain tissues using PCR-based subtractive hybridization in 2003. NDRG2, NDRG1, NDRG3, and NDRG4 comprise the NDRG gene family [1] and share approximately 59–68% homology. Additionally, NDRG family members display over 92% homology between humans and mice.

The expression and cellular localization of NDRG2 were altered following exposure to different stresses, supporting the role of NDRG2 as a cellular stress sensor. Wang et al. found that NDRG2 expression was markedly upregulated in several cancer cell lines exposed to hypoxic conditions or similar stresses at both the mRNA and protein levels [9]. Hypoxia-inducible factor-1α (HIF-1α) can directly bind to hypoxia response elements (HREs) in the NDRG2 promoter, thus upregulating NDRG2 expression under hypoxia. Importantly, enforcing the expression of NDRG2 can strongly increase the apoptosis of cancer cells. Alternatively, NDRG2 can translocate from the cytoplasm to the nucleus under DNA damage stress. However, no explicit nuclear localization signal (NLS) sequence has been identified in the NDRG2 protein. Although NLSs are the most common type of nuclear import elements, other mechanisms may also be involved in NDRG2 translocation. For example, Liu et al. and Cao et al. confirmed that NDRG2 was upregulated by p53 or adriamycin (ADR) treatment [10, 11]. Thus, NDRG2 can translocate into the nucleus and increase p53-dependent cell apoptosis through the DNA damage repair mechanism. Furthermore, we found that NDRG2 expression was decreased in ADR-resistant breast cancer cells. However, NDRG2 rescue could promote ADR sensitivity through inhibiting proliferation and promoting cellular damage responses and apoptosis in a p53-dependent manner. Interestingly, we found that NDRG2 upregulated Bad expression by increasing its half-life, which is associated with p53 expression in mitochondria. Thus, NDRG2 promoted the therapeutic sensitivity of breast cancer cells in a p53-dependent manner by preventing p53 from entering the nucleus to participate in DNA damage repair rather than by changing its expression [12].

We first found that NDRG2 is positively regulated by p53. The first intron of the NDRG2 gene contains a site that binds p53 directly and mediates wild-type (WT) p53-dependent transactivation [11]. In addition, NDRG2 enhances p53-mediated apoptosis, whereas overexpression of NDRG2 suppresses tumor cell growth independently of p53 mutation. NDRG2 enhances p53-mediated apoptosis of hepatocarcinoma cells by downregulating ERCC6 (also named cockayne syndrome B—CSB) expression, which is critical for nucleotide excision repair capacity [10]. Thus, excision repair cross-complementing complementation group 6 (ERCC6) is an NDRG2-inducible target gene that is involved in the p53-mediated apoptosis pathway.

3. NDRG2 functions as a novel ER stress-responsive protein and unfolded protein response (UPR) modulator

The ER is an essential organelle involved in many cellular processes, including protein folding and maturation, lipid synthesis and calcium homeostasis. When

cells are challenged by different environmental or intracellular insults, such as energy or nutrient deficiency, hypoxia, or oxidative stresses, ER's function is disrupted, causing accumulation of unfolded or misfolded proteins in the ER, a condition which is defined as ER stress [13, 14]. This triggers integrated signaling pathways to deal with the unfolded proteins, a phenomenon known as the UPR, which operates to restore ER homeostasis or, alternatively, lead to cell death under prolonged or severe ER stress [13, 14].

The UPR contains three branches initiated by three ER-resident transmembrane sensors: protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6) [15]. ER stress and the UPR are intensively involved in not only physiological conditions but also the pathogenesis of many diseases, including cancer [14, 16]. Accumulating implicates ER stress and the UPR in different aspects of tumorigenesis and tumor progression.

NDRG2 is a stress-responsive gene [1], and our laboratory recently reported that as such, NDRG2 is implicated in ER stress [17] in addition to the hypoxia and DNA damage response. Different ER stress inducers, including thapsigargin (Tg), tunicamycin (Tm) and dithiothreitol (DTT), can induce NDRG2 mRNA and protein expression in human hepatoma SK-Hep-1 and HepG2 cells. In NDRG2-overexpressing hepatoma cell lines and Ndrg2 knockout (KO) mouse liver tissues, among the three UPR branches, NDRG2 interacts with PERK upon ER stress and facilitates PERK pathway activity, enhancing downstream ATF4 and CHOP activity. Thus, overexpression of NDRG2 promotes ERS-induced apoptosis, while silencing or knockdown of NDRG2 does the opposite, both in cell lines and in vivo. These data suggest that NDRG2 is a novel ER stress-responsive protein and an important component of the UPRosome, acting as a PERK cofactor to facilitate PERK branch signaling and thereby contributing to ER stress-induced apoptosis [17]. Therefore, apart from its already established role, NDRG2 could be considered a component of the UPRosome and a key player in cell fate decisions during ER stress. However, whether NDRG2 regulates PERK by affecting its dimer/oligomer status or its post-translational modification or by competing with other regulators for binding is worthy of further investigation.

4. NDRG2 as a novel prognostic biomarker in cancer

NDRG2 expression is mainly detected in the muscle, brain, heart, liver, colon [18]. Interestingly, NDRG2 expression is nearly undetectable in the thymus, the bone marrow, the testis, and peripheral blood leukocytes, suggesting an inverse correlation between the NDRG2 gene expression level and cell proliferation status [18–20]. We and other groups confirmed the pattern of decreased NDRG2 expression in tumors compared with normal tissues in cancers including glioma [2, 19, 21], colorectal cancer [8, 22, 23], breast cancer [3, 24], lung cancer [25], thyroid cancer [26, 27], myeloid leukemia [28, 29] oral squamous cell carcinoma (OSCC) and cervical cancer [5, 7]. Collectively findings from these studies indicate that NDRG2 expression is decreased in most tumors. Moreover, NDRG2 expression was positively correlated with tumor differentiation but negatively correlated with lymph node metastasis and TNM stage (**Figure 1**).

We used a hospital-based study cohort of 226 colorectal cancer patients to analyze the correlation of *NDRG2* mRNA levels with the tumor clinicopathologic features, disease-free survival, and overall survival of colorectal cancer patients. *NDRG2* mRNA expression was significantly correlated with differentiation status, lymph node metastasis, and tumor-node-metastasis stage [23]. Patients with reduced NDRG2 mRNA levels had significantly worse progression-free survival (PFS) and

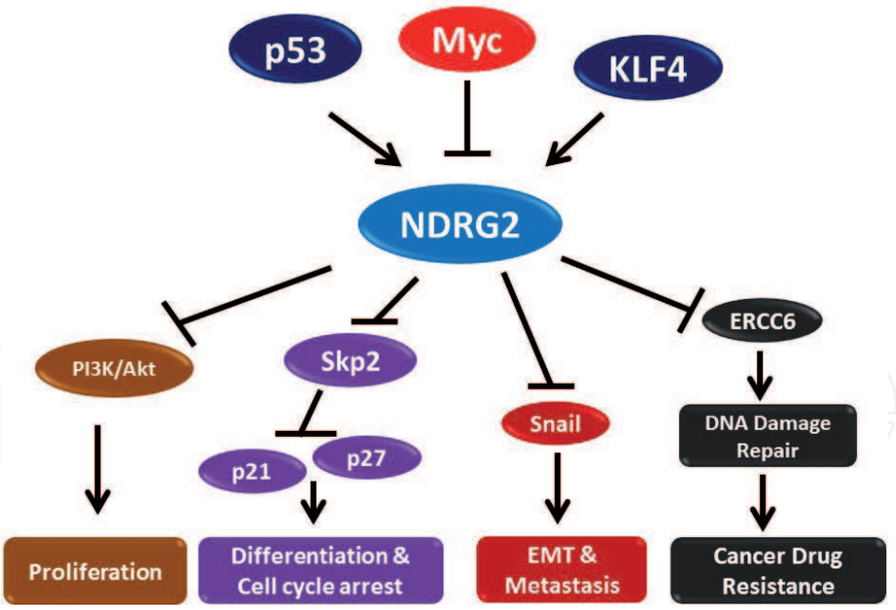

Figure 1.
The molecular working model of NDRG2. NDRG2 can be transcriptionally upregulated by p53 and KLF4, and repressed by Myc. NDRG2 inhibited cancer cells proliferation through blocking PI_3K/Akt signaling, promoted colorectal cancer cells differentiation through decreasing SKP2 and increasing p21/p27 expression, inhibited EMT through Snail abrogation, and sensitized cancer cells to chemotherapy with DNA damage repair inhibition.

overall survival (OS) than patients with preserved expression of NDRG2 mRNA. We provided the first evidence that the NDRG2 mRNA level is a novel independent prognostic biomarker for both PFS and OS in colorectal cancer patients [23].

Another study analyzed NDRG2 expression in 127 bladder cancer patients and 97 healthy controls. Similar to the findings in colorectal cancer, NDRG2 expression was significantly downregulated at both the mRNA and protein levels in the urine of patients with bladder cancer and was independently correlated with tumor grade and stage [30]. Thus, NDRG2 expression was decreased in patients with bladder cancer and might be a potential independent diagnostic biomarker for bladder cancer.

5. NDRG2 and differentiation

Differentiation deficiency is a key characteristic of cancer. Poorly differentiated cancers show high proliferation and metastasis capacities, which seriously impact patient survival and prognosis [31]. As a member of the human NDRG gene family, the involvement of NDRG2 in the regulation of cell differentiation has been fully addressed. Bioinformatics analysis of NDRG2 revealed several binding sequences for different transcription factors, which are mostly involved in growth regulation and early differentiation.

As a master switch for cell proliferation and differentiation, Myc performs its biological functions mainly through transcriptional regulation of its target genes, which are involved in cell interaction and communication with their external environment [32, 33]. We first provided the evidence that NDRG2 is transcriptionally repressed by Myc [34]. In addition, c-Myc overexpression dramatically reduced NDRG2 protein and mRNA levels. The core promoter region of NDRG2 is required for Myc-mediated repression of NDRG2 transcription, and the interaction of Myc with the core promoter region was verified both in vitro and in vivo. A mechanistic study showed that Miz-1 is involved in Myc-mediated NDRG2 repression, and is possibly through the recruitment of other epigenetic factors, such as histone deacetylases, to the promoter.

In colorectal cancer, the vast majority of poorly differentiated cells contain constitutive activation of WNT/β-catenin signal. WNT signaling-activating truncation mutations in adenomatous polyposis coli (APC) induce the nuclear translocation of β-catenin is induced, and consequently contributes to cell-fate determination *via* β-catenin/TCF complexes [35–38]. GSK-3β phosphorylates β-catenin at critical serine and threonine residues in its N terminus, which earmarks β-catenin for ubiquitination by the SCF complex and for subsequent degradation by the proteasome pathway [39, 40]. GSK-3β inactivation by APC mutation or oncogenic PI3K/AKT activation leads to the β-catenin/TCF complex formation, and further induced TCF target gene expressions, such as Myc, cyclin D1 [41, 42]. NDRG2 suppresses β-catenin nuclear translocation and decreases the occupancy of β-catenin/TCF complex on the promoter of E3 ligase Skp2, potentially through dephosphorylating AKT and GSK-3β. NDRG2-mediated suppression of Skp2 contributes to the induction and stabilization of p21 and p27, which are target proteins for Skp2-mediated degradation. Thus, NDRG2-meidated induction of cell differentiation is dependent on suppressing the activity of the Skp2 E3 ligase. In support of the biological significance of the reciprocal relationship between NDRG2 and Skp2, an $NDRG2_{low}$/$Skp2_{high}$ gene expression signature correlates with poor patient outcome and could be considered as a diagnostic marker for colorectal cancers.

Additionally, other groups have provided evidence of NDRG2 involvement of cell differentiation induced by different transcription factors, such as Wilms' tumor gene 1 (WT1) protein, HIF-1α and glucocorticoids [33, 54, 55]. Through an oligonucleotide array approach, WT1 was found to indirectly or directly induce the expression of NDRG2 mRNA in CD34+ cells and in leukemic U937 cells through an [54]. Moreover, a novel start site for NDRG2 expression appeared to be used in WT1-transduced cells, suggesting that this promoter is utilized preferentially when high levels of WT1 are present [54].

6. NDRG2 inhibits EMT and cancer metastasis

Metastasis is a unique feature of tumor cells and an important factor affecting the survival and prognosis of cancer patients; it is also an important reason that surgery cannot completely remove tumor lesions. EMT is an important process preceding tumor metastasis [43, 44]. During EMT, tumor cells change from an epithelioid morphology to a mesenchymal cell morphology. The adhesion abilities between cells were decreased [45, 46]. Various signaling pathways were found involved in the regulation of EMT, such as, TGF-β pathway [47], Wnt/β-catenin pathway [48] and Notch pathway [49].

Data indicate that NDRG2 is negatively regulated by TGF-β during the progression of hepatocellular carcinomas [6]. This observation may be due to impairment in the TGF-β/Smad signaling pathway or the activation of non-Smad signaling cascades (PI3K/AKT, p38MAPK and so on) in these cell lines in response to TGF-β. Accordingly, related evidence has shown that the enhancement of GSK-3β activity by NDRG2 overexpression causes proteasomal degradation of the Snail transcription factor and subsequent transcriptional regulation of EMT-related genes [50]. Thus, the tumor suppressor NDRG2 could inhibit TGF-β-induced EMT as well as cell invasion and migration in various cancers. Similarly, a study showed the inhibitory effect of NDRG2 on TGF-β-induced tumor metastasis *via* the attenuation of active autocrine TGF-β production [51].

In breast cancer, NDRG2 downregulated the expression of Snail, as well as the phosphorylation of signal transducer and activator of transcription 3 (STAT3), an oncogenic transcription factor activated in many human malignancies, including

breast cancer [24]. Further, NDRG2 overexpressing breast cancer cells showed markedly decreased Snail expression after treatment with STAT3 inhibitors. Thus, the inhibition of STAT3 signaling by NDRG2 suppresses EMT progression *via* the down-regulation of Snail expression. Moreover, high NDRG2 expression induced inactivation of NF-κB and PI3K/AKT signaling pathways *via* the dephosphorylation of the C-terminal domain of PTEN, and the inhibition of the EMT process in OSCC [7]. Therefore, NDRG2 may regulate tumor EMT *via* different regulatory mechanisms in different cancers.

7. NDRG2 is involved in cancer metabolism by regulating glycolysis, glutaminolysis and fatty acid oxidation (FAO)

A cancerous cell undergoes multiple steps to form a solid tumor entity, during which nutrient and oxygen supply insufficiencies frequently occur. In recent decades, studies have provided deep insight into cancer metabolism. In addition to glycolysis, metabolic alterations involve almost all metabolic pathways, including those of lipids, amino acids, nitrogen, and nucleic acids. Metabolic reprogramming is widely accepted to be a hallmark of cancer [52]. Cancer metabolic reprogramming has been further summarized into six hallmarks, including alterations in nutrient uptake (deregulated uptake of glucose and amino acids and the use of opportunistic modes of nutrient acquisition) and intracellular metabolic pathways (the use of glycolysis/TCA cycle intermediates for biosynthesis and NADPH production and an increased demand for nitrogen) [53]. For instance, cancer cells use glucose and glutamine as the major sources of energy and precursor intermediates, thus exhibiting enhanced glycolysis and glutaminolysis [53]. Under various stress conditions, such as, glucose deficiency, cancer cells can shift from glycolysis to FAO to maintain ATP levels and satisfy nutrient demands [54]. Not surprisingly, oncogene activation and tumor suppressor inactivation are extensively involved in these processes. For example, c-Myc, HIF-1α, and p53 can regulate the uptake of both glucose and glutamine and glycolytic flux by affecting the expression of glucose transporters and metabolic enzymes [53].

As a tumor suppressor, NDRG2 was found to regulate aerobic glycolysis and glutaminolysis in cancer cells. A previous study from our laboratory showed that NDRG2 inhibits glucose uptake by interacting with and promoting the degradation of glucose transporter 1 (GLUT1) without affecting its transcription in breast cancer cell lines [55]. Recently, Xu et al. [56] from our laboratory, using colorectal cancer cells and a xenograft model, also demonstrated that NDRG2 inhibits glucose uptake and glycolysis by suppressing the expression and activity of the glucose transporter GLUT1 and key glycolytic enzymes, including hexokinase 2 (HK2), pyruvate kinase M2 isoform (PKM2) and lactate dehydrogenase A (LDHA). In addition, NDRG2 inhibits glutaminolysis by suppressing the expression of the glutamine transporter ASC amino acid transporter 2 (ASCT2) and glutaminase 1 (GLS1) at the transcriptional level. Mechanistically, NDRG2 exerts such effects by suppressing the expression of β-catenin, leading to the repression of its target gene c-Myc. Since c-Myc is a master regulator of metabolism, additional in-depth studies on NDRG2's regulatory role in other tumor glucose catabolism pathways are needed.

Under stress conditions such as glucose limitation, FAO is always activated to preserve the supply of ATP and NADPH [54]. Interestingly, our most recent study [4] revealed that NDRG2, as a negative regulator of AMPK, suppresses glucose deprivation-induced activation of the AMPK/ACC pathway and the consequent induction of FAO genes in hepatoma cells. Thus, NDRG2 overexpression leads to dysregulation of ATP and NADPH, thereby reducing the tolerance of hepatoma

cells to glucose limitation. Together, these data further our understanding of the tumor-suppressive mechanism of NDRG2 through its involvement in cancer metabolic reprogramming. Therefore, the application of NDRG2 alone or in combination with antiglycolytic agents such as 2-diacylglycerol (2-DG) may effectively and synergistically inhibit cancer cells, which rely heavily on either glycolysis under non-stressful conditions or FAO under conditions of metabolic stress.

8. NDRG2 knockout enhances tumorigenesis in vivo

Most of the evidence for the role of NDRG2 as a tumor suppressor was mainly obtained in vitro, and establishing an in vivo mouse model to confirm these findings was crucial. It is reported that *Ndrg2*-deficient mice are susceptible to spontaneous tumor formation in vivo and *Ndrg2* knockout mice developed various types of tumors, including lymphomas, hepatocellular carcinomas and bronchoalveolar carcinomas [28]. However, we did not replicate these findings in our established Ndrg2 knockout mouse model—indeed, we did not detect any tumorigenesis in mice at 24 months of age. This discrepancy might be due to the different mouse strains and knockout strategies.

Notably, we established intestine-specific *Ndrg2* knockout mice using a Villin-Cre; $Ndrg2^{flox/flox}$ strategy [57]. Intestinal *Ndrg2* deficiency significantly augmented colitis initiation and colitis-associated tumor development. Ndrg2 loss led to the destruction of adherens junction structure *via* E-cadherin reduction, resulting in diminished epithelial barrier function and enhanced gut permeability. We identified the novel mechanism by which NDRG2 is crucial for the interaction of the E3 ligase FBXO11 with Snail, the repressor of E-cadherin. Thus, Ndrg2 loss increased Snail protein stability and decreased E-cadherin expression (https://www.biorxiv.org/content/10.1101/473397v1). Moreover, our study revealed that NDRG2 is an essential intestinal epithelial barrier regulator and plays important roles in gut homeostasis maintenance and colitis-associated tumor development.

Recently, we established a liver cancer metastasis model in WT and Ndrg2 knockout ($Ndrg2^{-/-}$) mice and found that expression loss of the tumor suppressor Ndrg2 in the liver microenvironment significantly suppressed the growth of liver cell colonies [57, 58]. Our data highlighted the role of NDRG2 in the regulation of tumor-associated macrophage (TAM) polarization and its function in promoting cancer liver metastasis. Interestingly, a reduced metastatic burden was correlated with an increased percentage of M1-like TAMs and decreased expression of M2-associated markers in the NDRG2-deficient microenvironment [58]. In summary, our study is the first showing a crucial and unexpected role for NDRG2 in macrophage polarization and highlights the importance of investigating the function of NDRG2 in cancer cells and the tumor microenvironment differently.

9. NDRG2 in brain tumors and other nervous system diseases

Accumulating studies have shown that NDRG2 is associated with various nervous system diseases, including tumors, ischemic stroke, hemorrhage, trauma, and neurodegenerative disorders [1, 59]. NDRG2 was repeatedly reported to be downregulated in a variety of cerebral tumors, including glioma and meningioma [21, 60–66]. The transcription levels of human *NDRG2* are significantly reduced in human glioblastoma tissues and human glioblastoma cell lines, and exogenous overexpression of NDRG2 repressed glioblastoma cell proliferation in vitro [2]. Although direct structural alterations such as point mutations are very rare in the

NDRG2 gene, hypermethylation of the *NDRG2* promoter region was shown to be highly correlated with decreased *NDRG2* transcription levels in human glioblastoma [60, 61, 67, 68]. In addition to the direct impact of *NDRG2* hypermethylation *per se*, NDRG2 may control glioma cell growth by upregulating the levels of histone acetylation in glioma cells [62]. Moreover, the expression level of *NDRG2* was negatively correlated with the pathological grade of the brain tumors and positively correlated with survival in astrocytoma patients [21, 63]. Consistent with the results in glioblastoma, a decrease in the levels of *NDRG2* gene methylation and NDRG2 protein expression were detected in human meningioma [64]. In addition, the expression levels of NDRG2 were significantly further reduced in recurrent meningioma compared to that in primary meningioma [65]. The above results suggest that NDRG2 may be a potential biomarker for predicting the prognosis of human brain tumors.

NDRG family members are abundantly expressed in brain tissue; therefore, the significant functions of these NDRG2 family members in the central nervous system were anticipated and have been confirmed with *NDRG* gene knockout mice-based studies [69–71]. NDRG1 deficiency leads to a progressive demyelinating in the peripheral nerves, suggesting that *NDRG1* is involved in the maintenance of and axonal survival and myelin sheath structure [69]. *Ndrg2*$^{-/-}$ mice exhibited typical ADHD-like behaviors, including hyperactivity, impulsivity, and inattention, as well as impaired memory [70]. *Ndrg4*$^{-/-}$ mice showed impaired cognition and increased susceptibility to ischemic stroke, indicating that NDRG4 has a potential neuroprotective effect [71].

In addition, NDRG2 was implicated in the ischemic stress response in several in vivo and in vitro studies [72–78]. Temporal and spatial patterns of NDRG2 expression in the rat brain were investigated after transient middle cerebral artery occlusion and reperfusion. Both the mRNA and protein levels of NDRG2 were increased following reperfusion in the ischemic penumbra, and NDRG2 was translocated from the cytoplasm to the nucleus in astrocytes. Moreover, NDRG2 expression increased in parallel with the enhancement of TUNEL signals in this ischemic animal model [73]. It is consistent with the results of the animal experiments described above, the expression of NDRG2 was also revealed to be upregulated and NDRG2 can translocate from the cytoplasm to the nucleus in C6-originated astrocytes after oxygen-glucose deprivation (OGD) treatment mimicking ischemic model in vitro [72]. Furthermore, NDRG2 was implicated in some types of cerebral ischemic preconditioning-mediated neuroprotection, including electroacupuncture (EA) [75] and sevoflurane preconditioning [74]. EA preconditioning in the Baihui acupoint was performed before transient focal cerebral ischemia and reperfusion. After EA pretreatment, the number of apoptotic cells in the ischemic penumbra and the volume of cerebral infarct were significantly decreased, and the neurological outcomes were effectively rescued. After ischemia treatment, the levels of NDRG2 expression were largely suppressed in the EA pretreatment group compared with sham group. And NDRG2 was mostly localized in the astroglial cytoplasm; only weak staining was found in the astroglial nucleus after EA pretreatment. However, NDRG2 protein was remarkably transferred from the cytoplasm into the nucleus in the sham group [75]. Recently, NDRG2 was also found to exhibit neuroprotective effects with sevoflurane preconditioning in brain ischemia models both in vivo and in vitro [74]. These results together indicate that NDRG2 takes part in the pathological process of brain ischemia-reperfusion injury and that NDRG2 may be a potential intervention target for ischemic stroke.

NDRG2 has also been repeatedly reported to be associated with other nervous system diseases, such as, neurodegeneration [79–81] and depression [82–84]. NDRG2 has been identified as one of six aberrantly phosphorylated proteins in

human brains with frontotemporal lobe degeneration, and an increased phospho-spectra of NDRG2 was found in these neurodegenerative tissues [85]. Accumulated NDRG2 and GFAP were detected in cortical senile plaques from the postmortem human brain tissues with Alzheimer's disease (AD) [79]. In addition, the expression levels of NDRG2 and GFAP were parallelly increased in amyloid precursor protein (APP)/presenilin (PS1) mouse, a double transgenic AD mouse model [80]. Suppressed NDRG2 expression and decreased memory impairment were detected in parallel after EA treatment to APP/PS1 transgenic mice. Furthermore, the increased reactive astrocytes andNDRG2 expression were detected in the mice which were exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a Parkinson's disease-associated neurotoxin that causes both glial activation and neurodegeneration [86]. Moreover, growing studies have demonstrated that NDRG2 is related with the function of antidepressants, which can correct depression-like behaviors and alleviate neural damages observed in depressive animals [82–84]. NDRG2 was downregulated in the rat frontal cortex after chronic use of antidepressants [84]. In contrast to the results described above antidepressants did not counteract the increase in NDRG2 expression in the hippocampus of rats with stress-induced depression-like symptoms and that antidepressants *per se* induced NDRG2 expression in normal rats [83]. Further study of the detailed mechanisms by which NDRG2 participates in these neurodegenerative or chronic psychiatric diseases providing novel intervention strategies will thus be interesting.

10. Conclusion and perspectives

To date, both in vitro and in vivo evidence has shown that NDRG2 can inhibit cancer cell proliferation, EMT, metastasis and can promote cell differentiation and cell cycle arrest. Thus, NDRG2 might be a target for cancer treatment and therapeutic resistance. Although NDRG2 is a novel tumor suppressor, the detailed mechanism by which NDRG2 functions requires further elucidation. Moreover, additional in vivo data are needed to confirm its tumor suppressor function.

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