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Review

NDRG2: a Myc-repressed gene involved in cancer and cell stress

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As a master switch for cell proliferation and differentiation, Myc exerts its biological functions mainly through transcriptional regulation of its target genes, which are involved in cells' interaction and communication with their external environment. The N-Myc downstream-regulated gene (*NDRG*) family is composed of *NDRG1*, *NDRG2*, *NDRG3* and *NDRG4*, which are important in cell proliferation and differentiation. This review summarizes the recent studies on the structure, tissue distribution and functions of *NDRG2* that try to show its significance in studying cancer and its therapeutic potential.

Keywords N-Myc downstream-regulated gene; tumor suppressor; cell stress

MYC was among the earliest oncogenes identified and has been the subject of intensive study in recent years. There has been a strong driving force to explore the function and regulation of Myc in cell proliferation, differentiation and apoptosis as well as its role as an important transcriptional factor [1].

As a master switch for cell proliferation and differentiation, Myc exerts its biological functions mainly by transcriptional regulation of its target genes [2]. The identification of authentic Myc target genes, both direct and indirect, is important in understanding the mechanisms it uses to regulate cell behavior [3]. A significant fraction of Myc-repressed genes are involved in cells' interaction and communication with their external environment. It is especially interesting to note that several of these targets have been shown to possess tumor suppressor and anti-metastatic properties [4].

N-Myc downstream-regulated gene 2 (*NDRG2*) [5–7], together with *NDRG1* [8–11], *NDRG3* [6,12], and *NDRG4* [13,14], constitute the *NDRG* gene family, a new class of Myc-repressed genes. Accumulated data have shown the importance of this gene family in cell proliferation and differentiation. Significant attention has been paid to the *NDRG* gene family due to its potential as a tumor suppressor as well as its involvement in other diseases. Kovacevic *et al* and Ellen *et al* reviewed *NDRG1*'s structure, regulation of gene expression, and function in normal and disease states [15,16]. This review focuses primarily on *NDRG2*, a member of the *NDRG* gene family that, like *NDRG1*, has been significant in cancer studies and has shown therapeutic potential. Comparisons of the structure and function of different members of the *NDRG* family are also discussed.

Gene and Protein Structure of *NDRG2*

Shimono *et al* first used "*NDRG*" to describe a gene that was identified as representative of those that expressed higher N-Myc in the knockout mouse embryos [11]. The *NDR1* (murine *NDRG1*) gene was augmented 20-fold in the mutant embryos at 10.5 d post coitus, which is indicative of repression by N-Myc. A negative correlation is shown between the expression of N-Myc and *NDRG1* in various developing tissues of the wild-type embryos. The same group identified *NDRG2* and *NDRG3*, which encode proteins highly related to *NDRG1*. However, *NDRG2* and *NDRG3* are under spatio-temporal regulations that differ from *NDRG1* [6], which are not activated in N-*Myc* mutants.

Human *NDRG1* gene was actually discovered earlier than the mouse *NDRG* family, but it was given a different name and lacked recognition as a downstream-regulated gene of Myc protein. In 1997, Kokami *et al* discovered a gene in which expression was induced by reducing agent and tunicamycin in human umbilical vein endothelial cells

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[8]. The gene was termed as *RTP*, standing for reducing agent and tunicamycin responsive protein. Van Belzen *et al* cloned this same gene and called it *DRG1* (differentiation related gene) [9]. They found that *DRG1* expression decreased in colon adenoma and adenocarcinomas, but it increased 20-fold when colon cancer cell differentiation was induced.

Deng *et al* first described the human *NDRG2* sequence as a protein containing an acyl-carrier protein (ACP)-like domain [17]. The gene was cloned by polymerase chain reaction-based subtractive hybridization from glioblastoma, using normal brain tissues as control. Other members of the human *NDRG* gene family were reported later (**Table 1**). The human *NDRG* family consists of four members: *NDRG1*, *NDRG2*, *NDRG3* and *NDRG4* [5–14]. Some alternative splicing isoforms exist in *NDRG2* and *NDRG4* [13,18,19].

Members of the *NDRG* family exist in many species, including zebrafish [20], *Xenopus laevis* [21], *Drosophila melanogaster*, *Caenorhabditis elegant* and *Dictyostelium discoideum* as well as in plants, such as sunflowers [22]. However, there is no protein with a significant similar sequence to NDGR2 in procaryotes, fungi and protozoa, which means that the distribution of *NDRG* family members is restricted to metazoa and plants.

Although *NDRG* members do not possess a clear functional peptide motif, they do share some well conserved residues. The percentage of shared residue identities among members is approximately 60% (**Fig. 1**) [13]. Phylogenetic analysis revealed that *NDRG1* and *NDRG3* belong to one subfamily, whereas *NDRG2* and *NDRG4* belong to another [7].

The Joint Center for Structural Genomics presented the partial crystal structure (residue 40–313) of mouse NDRG2 to the RCSB Protein Data Bank (2QMQ) (http://www.pdb.org/pdb) [23]. It contains 15 helices and 10 strands. Two domains can be recognized in the construct, an α/β hydrolase catalytic domain and a cap domain. Although the structure analysis grouped NDRG2 protein into α/β hydrolase superfamily, the key motif of catalytic activity in hydrolase seems absent. The catalytic triad in a specific order (nucleophile-acid-histidine), such as Ser-His-Asp/Glu triad motif, which exists in other hydrolase members [24], is not found in the structure of NDRG2 using multiple sequence alignment.

NDRG2 contains several potential phosphorylation sites and has been confirmed to be phosphorylated in certain cells by different protein kinases [25–27]. NDRG2 phosphorylation was increased in C2C12 cells (mouse myoblast cell line) co-overexpressing either PKC or Akt and stimulating insulin in a wortmannin- and palmitate-inhibitable manner, supporting a direct role for Akt. Thr348 is the major phosphorylation site for Akt, whereas PKC phosphorylates Ser332 [25]. NDRG2 phosphorylation was also observed in rabbit skeletal muscle extracts. It was phosphorylated rapidly by serum and glucocorticoid-induced kinase 1 (SGK1), but not by Akt-α. SGK1 phosphorylated NDRG2 at Thr330, Ser332 and Thr348 *in vitro* [26]. All three residues were phosphorylated in skeletal muscle from wild-type mice, but not from mice that do not express SGK1. NDRG1 was also phosphorylated by SGK1 at Thr328, Ser330 and Thr346 (equivalent to NDRG2's Thr330, Ser332 and Thr348) as well as at Thr356 and Thr366. Interestingly, Thr356 and Thr366 are located within identical decapeptide sequences, GTRSRSHTSE, which is repeated three times in NDRG1 but absent in other members of the NDRG family. These threonines were phosphorylated in NDRG1 in the liver, lung, spleen and skeletal muscle of wild-type mice, but not in $SGK1^{-/-}$ mice. The phosphorylation of NDRG1 by SGK1 transformed it into an excellent substrate for glycogen synthase kinase 3, which could then phosphorylate the Ser342, Ser352 and Ser362 of NDRG1 in the repeat region [26]. Furthermore, the phosphorylation of

Table 1 The comparison of human N-Myc downstream-regulated gene (*NDRG*) gene family

Member	Chromosome location	Number of exon	Number of amino acid coding
NDRG1	8q24	16	394
NDRG2	14q11.1-11.2	14	371
NDRG2^{var}	14q11.1-11.2	13	357
NDRG3	20q11.21-11.23	16	375
*NDRG4*B	16q21-22.1	15	339
*NDRG4*B^{var}	16q21-22.1	16	352
*NDRG4*H	16q21-22.1	17	371

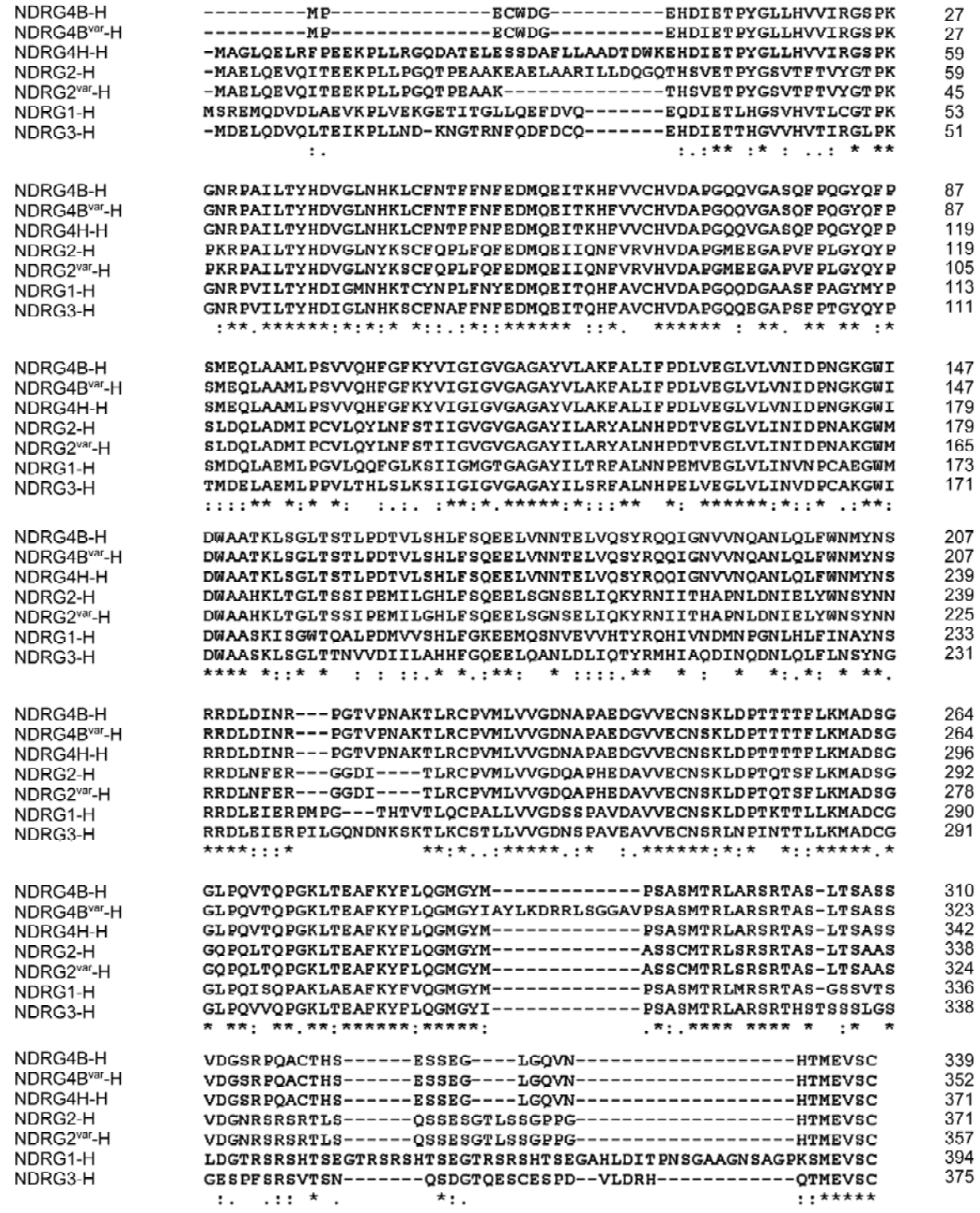

Fig. 1 Comparison of the amino acid sequences of the human N-Myc downstream-regulated gene (*NDRG*) family The asterisk (*) represents the complete identity among each member, and the period (.) and the colon (:) represent the partial consistency.

NDRG2 at T330 and T334 were detected by MALDI-TOF/TOF and nano-LC-ESI-MS/MS analysis of hippocampus protein extract from rats [27].

Tissue Distribution of NDRG2

The expression pattern of human *NDRG2* gene in various cells and tissues were first analyzed at the mRNA level [5, 7]. Northern blot analysis using a human RNA master blot revealed that *NDRG2* exists as a 2.0 kb single transcript. Prominent hybridization signals were detected in the muscle, brain, heart, liver and, to a lesser extent, in the kidney. In adult tissues, the highest expression level was found in the salivary glands, various neural tissues and skeletal muscles [5]. Furthermore, *NDRG2* mRNA is nearly undetectable in the thymus, bone marrow, testis and peripheral blood leukocyte. This expression pattern suggests an inverse correlation between the level of *NDRG2* gene expression and the rate of cell proliferation. In addition, the *NDRG2* transcript was not detected in any of the tumor cell lines examined. These cell lines included leukemia (HL-60, K-562, and MOLT-4), lymphoma (Burkitt's lymphoma I, Raju and Daudi), lung carcinoma (A549), and colorectal carcinoma (SW480) [5].

Regarding the cellular and tissue distribution of NDRG proteins, human NDRG1 protein was first reported to be found mostly in epithelial cells [28]. Later, Wakisaka *et al* described rat NDRG1 protein expression in the kidney and brain [29]. The localization of NDRG1 protein in the kidney changed from the proximal convoluted tubules to the collecting ducts between 10 d and 20 d postnatal. In the brain, a change in cellular expression was also found from the hippocampal pyramidal neurons to the astrocytes in the gray matter during the same postnatal period.

Hu *et al* used an anti-NDRG2 monoclonal antibody to analyze the expression pattern of NDRG2 protein in mouse embryos at various gestational ages and in a variety of adult mouse tissues [30]. NDRG2 immunostaining was generally localized to the cytoplasm. During mouse development, NDRG2 expression was observed in many developing tissues and organs, including the heart, brain, lung, gut, liver, kidney, skeletal muscle, cartilage, chorion, epidermis and whisker follicles. NDRG2 expression was generally lower in the early stages of development and markedly increased during later stages. NDRG2 protein was also observed in a variety of adult mouse tissues, particularly in the heart and brain [30].

NDRG1 and NDRG2 protein expression has remarkable differences. NDRG1 protein commonly exists in various epithelia, including glandular epithelium [28], whereas NDRG2 is not detected in most epithelia, including glandular epithelium [30]. In terms of organ distribution, a differential expression between NDRG1 and NDRG2 has been noted in the heart, brain, testicles, ovaries and uterus. For instance, NDRG2 protein is highly positive throughout cardiac muscle [30], whereas NDRG1 protein is not expressed at all despite the existence of its mRNA [28]. NDRG2 is distributed widely in the brain, especially at high levels in the midbrain, cerebellum, medulla and thalamus [31], whereas NDRG1 is mainly found in the cortex and hippocampus [29].

Okuda *et al* recently made a precise comparison of the distribution of the NDRG family of proteins in the central nervous system [32]. They demonstrated that NDRG1 and NDRG2 were localized in the oligodendrocytes and the astrocytes, respectively, in the cerebrum. In the cerebellum, NDRG1 and NDRG4 were localized in Purkinje cells, while NDRG2 was in Bergmann glial cells. Shen *et al* further confirmed the NRDG2 protein's localization in the astrocytes by glial fibrillary acidic protein co-staining [31]. Interestingly, NDRG2 expression increased while the glioma cells were differentiating into astrocytes. These expression patterns revealed the cell type-specific and ubiquitous localization of the NDRG family of proteins. Each NDRG member may play partially redundant roles in specific cells in the brain [32].

In most of the reports, NDRG2 protein exists primarily in the cytoplasm [5,7,28,31,32], but it is also associated with the cell membrane and adherens junctions [28], even in the nuclei [30,31]. The nuclear staining of NDRG2 was initially observed in some cells in the midbrain of mice [30], and then confirmed in cells with astrocyte-like morphology in the hippocampus and cerebral cortex as well as in the olfactory bulb tissue [31]. Okuda *et al* found that NDRG3 was detected in the nuclei in most cells [32]. More importantly, NDRG1 and NDRG2 were translocated into nuclei upon cell stress [34,35]. The NDRG1-heat shock cognate protein 70 complex also transiently appeared in the nuclear fraction of activated mast cells [35].

The NDRG members have different tissue expression patterns, indicating that they may play distinct roles. More distribution information of NDRG proteins, especially in human tissues, is needed to better understand their function. The mechanisms and the conditions of nuclear localization of NDRG1 and NDRG2 proteins should be investigated more extensively.

NDRG2 Alterations in Human Cancer

The various *NDRG* family members are reportedly intimately involved in cellular differentiation and development. *NDRG1* has been associated with differentiation [9], embryo development [11], and tumor suppression [36,37]. Reduced expression of NDRG1 has been implicated in cancer cell proliferation and metastasis [15,16,34,36–40].

Given the fact that *NDRG2* was cloned as a down-regulated gene in glioblastoma [17], several research groups investigated and confirmed its differential expression between tumor and normal tissues [5,41–48]. Deng *et al* analyzed *NDRG2* mRNA levels in six normal brain tissues, 27 cases of human glioblastoma, 13 cases of low-grade glioma (from grade I to grade III), and six human high-grade astrocytoma/glioblastoma cell lines [5]. Their results demonstrated that the expression of *NDRG2* was significantly reduced in 56% of human glioblastoma tissue samples and 100% of cell lines, as compared to expression in the normal brain or the low-grade glioma samples.

Lusis *et al* found that NDRG2 expression was consistently down-regulated in grade III meningioma at both the transcript and protein levels in independent sets of clinically and pathologically diverse cases [41]. Loss of *NDRG2* expression was also seen in a subset of lower-grade meningiomas, including atypical meningiomas (WHO grade II) with clinically aggressive behavior. In the Norwegian cohort, mRNA levels of *NDRG2* were significantly reduced in colorectal carcinoma when compared with those in the healthy controls. There was a trend for a decrease in *NDRG2* levels with increasing Dukes' stage [42]. Choi *et al* demonstrated that only two gastric cancer cell lines, SNU-16 and SNU-620, expressed *NDRG2* among seven gastric cancer and two non-cancer cell lines [43]. *NDRG2* was highly expressed in normal gastric tissues, but gastric cancer patients were divided into *NDRG2*-positive and *NDRG2*-negative groups. The survival rate of *NDRG2*-negative patients was lower than that of *NDRG2*-positive patients. It was confirmed that the loss of *NDRG2* expression was a significant and independent prognostic indicator in gastric carcinomas by multivariate analysis [43].

Other groups also reported decreased *NDRG2* gene expression in different cancer tissues, such as breast cancer [44], liver cancer [45], gastric cancer [46], oligodendroglial tumours [47], and skin cancer [48].

Hypermethylation is one of the most important attributes in the down-regulation of the *NDRG2* gene. The loss of *NDRG2* expression was significantly associated with hypermethylation of the *NDRG*2 promoter [41] in meningioma and several breast cancer cell lines [44]. In addition, the heterozygous deletion of *NDRG2* was confirmed in breast cancer cell line MCF-7. It was also noticed that mutation [−13 bp (C>T)] of the *NDRG2* core promoter significantly reduced *NDRG2* activity *in vitro* [44].

While there have yet to be any controversial results reported about *NDRG2* reduction in a variety of cancers, the same is not true of *NDRG1*. Though most research has reported down-regulation of *NDRG1* expression in colon, breast and prostate cancers, some studies have reported increased expression of NDRG1 protein in colon and prostate malignancy [49,50]. The explanation for the inconsistent results is that NDRG1 expression patterns may reflect the prostatic epithelium's varying responses to hypoxia and androgens in African-American and Caucasian patients [50].

Collectively, these data identify *NDRG2* as the candidate tumor suppressor gene and suggest that *NDRG2* may be a useful and functionally relevant biomarker for predicting aggressive forms of cancer.

A recombinant adenovirus designed to preferentially eliminate p53-negative cells has shown that the loss of tumor suppressor activity may be therapeutically exploited [51]. Based on *NDRG2*'s down-regulation in cancer tissue, several studies have followed it with interest to determine its potential as a target for cancer treatment. Most of these studies reported on the inhibitory effect of *NDRG2* overexpression on tumor malignancy. The transfection of human glioblastoma U373 and U138 cells with a complementary DNA encoding *NDRG2* was shown to markedly reduced cell proliferation [5]. *NDRG2*-silenced SNU-620 (gastric cancer) cells exhibited slightly increased proliferation and cisplatin resistance [43]. Additionally, inhibition of *NDRG2* decreased Fas expression and Fas-mediated cell death. The inhibition of cell proliferation by *NDRG2* overexpression in malignant cancer cells was also reported in other cancers, such as liver [52], lung [34], and breast cancers [44]. It was reported that *NDRG2* overexpression in malignant breast cancer cells specifically inhibits Akt phosphorylation and induces phosphorylation of p38 mitogen-activated protein kinase and SAPK/JNK. Meanwhile, JAK2 or STAT3 activation in both resting and IGF-stimulating cells was inhibited by *NDRG2* expression, implicating *NDRG2* as a growth inhibitory gene in signal transduction pathways of breast tumor cells [53].

Considering the similar inhibitory effect of *NDRG2* and *NDRG1* on tumor cells, it is worth testing these targets for cancer treatment using gene manipulation strategies as well as small chemical treatments.

Control of *NDRG2* Gene Expression by Myc and Other Factors

Bioinformatics analysis of *NDRG2* revealed several binding sequences for different transcription factors [54], which are mostly involved in growth regulation and early differentiation of cells. Some of those factors, such as Wilms' tumor gene 1 (*WT1*) protein, hypoxia-induce factor-1α

(HIF-1α) and glucocorticoids, up-regulate *NDRG2* expression [34,55,56]. HIF-1α also up-regulates *NDRG1* expression, whereas WT1 protein and glucocorticoids only regulate *NDRG2* expression.

WT1 protein is a transcriptional regulator that is highly expressed in immature hematopoietic progenitor cells and in the majority of patients with acute and chronic myeloid leukemia. A WT1 binding site exists upstream of the *NDRG2* promoter (–379 to –391 bp). Svensson *et al* found that WT1 indirectly or directly induced the expression of *NDRG2* mRNA in CD34+ cells and in leukemic U937 cells through an oligonucleotide array approach [55]. Morevoer, a novel starting site for *NDRG2* expression appeared to be used in WT1-transduced cells only, suggesting that this promoter is utilized preferentially when high levels of WT1 are present [55].

Sequence analysis of the human *NDRG2* gene promoter revealed three putative hypoxia-responsive elements (HRE) motifs: HRE-1 from –188 to –183 bp; HRE-2 from –371 to –367 bp; and HRE-3 from –377 to –373 bp. Wang *et al* provided evidence that the expression of *NDRG2* was regulated by HIF-1α in tumor cells upon hypoxia, and HRE1 could directly bind HIF-1α *in vivo* [34]. They suggested that HRE-1, HRE-2 and HRE-3 in the *NDRG2* promoter are closely related to the regulation of HIF-1α. HRE-1 is more important than HRE-2 and HRE-3 because the deletion or mutation of HRE-1 in the *NDRG2* promoter resulted in a dramatic decrease of luciferase activity induced by HIF-1α, whereas the deletion or mutation of HRE-2 and HRE-3 only resulted in a slight decrease of that activity.

Glucocorticoids reportedly up-regulate *NDRG2* gene expression in rat brains, though a specific binding site for glucocorticoids receptor has not been identified [56].

More detailed studies have been done about the up-regulation of *NDRG1* gene expression than *NDRG2*. *NDRG1* is controlled by several known cell differentiation reagents [38], such as ligands [e.g. peroxisome proliferator-activated receptor gamma (troglitazone and BRL46593) and retinoid X receptor (LG268)] and histone deacetylase inhibitors (e.g. trichostatin A, suberoylanilide hydroxamic acid). The expression of *NDRG1* was induced by DNA-damaging agents as well as by enforced expression of wild-type p53 [57,58]. The elevation of *NDRG1* expression in G_1 and G_2/M phases was believed to have resulted from p53-mediated transcription activation, which is known to cause cell cycle arrest upon DNA damage. In addition to p53, other transcription factors, such as HIF-1, c-Jun/AP-1, E2a-Pbx1 fusion protein [59], were also implicated in the regulation of *NDRG1* gene expression. HIF-1 is required for Ni^{2+} compound-induced *NDRG1* expression. C-Jun/AP-1 plays an important role in Ca^{2+} ionophore and iron chelator [60], as well as in hypoxia-induced *NDRG1* expression [39,61]. In addition, comparison of the noncoding sequence of the rat *NDRG4* gene [19] and human *NDRG2* gene with those of the orthologous mouse and human genes suggests that the AP-1 binding site is a candidate regulatory element.

As a downstream gene of Myc, mouse *NDRG1* expression was found to be repressed by N-Myc and c-Myc [11]. The *NDRG1* promoter activity was down-regulated by N-Myc, and more strongly by the combination of N-Myc and Max in the cotransfection assay [11]. This repressive effect was through the promoter region within 52 base pairs from the transcription start site. The effect of N-Myc:Max was sensitive to trichostatin A, indicating the involvement of histone deacetylase activity in repressing the *NDRG1* promoter [11]. In contrast, N-Myc does not seem to regulate mouse *NDRG2*, since the expression of *NDRG2* was not up-regulated in tissues of N-Myc knockout mice [6].

The evidence that human *NDRG1* is repressed by N-Myc overexpression in neuroblastoma cell lines suggests its similarity with mouse *NDRG1* with regard to gene expression regulation. Human *NDRG1* is strongly repressed in all tested neuroblastoma cell lines bearing N-*MYC* amplification, as well as in a neuroepithelioma line with amplified c-*MYC* [62]. *In vitro* interaction of Myc protein with the *NDRG1* core promoter was found in cells. The re-expression of *NDRG1* in high-*MYC* neuroblastoma cells resulted in smaller cells with reduced colony size in soft-agar assays, further underscoring the functional significance of *NDRG1* in human cancer cells [62].

Although N-Myc does not seem to regulate mouse *NDRG2*, an elevated c-*MYC* mRNA level was found in human glioblastoma with reduced *NDRG2* mRNA [5]. It suggests that human *NDRG2* might be regulated differently than mouse *NDRG2* as the target of Myc. Zhang *et al* provided evidence that the expression of human *NDRG2* is down-regulated by Myc via transcriptional repression [63]. The ectopic expression of c-Myc dramatically reduces the cellular NDRG2 protein and mRNA levels. Furthermore, this confirmed the core promoter region of *NDRG2* necessary for Myc repression on *NDRG2* transcription and verified the interaction of Myc with the core promoter region both *in vitro* and *in vivo*. Moreover, the c-Myc-mediated repression of *NDRG2* requires association with Miz-1 and possibly the recruitment of other epigenetic factors, such as HDACs, to the promoter [63]. Till now, Zhang's work concerning the transcriptional repression of *NDRG2* by Myc is the relative explicit

report about the mechanism research of *NDRG* family.

Compared with the sequences predicted to be regulated by various transcription factors in the promoter region of the *NDRG* gene family, only a few elements have been studied at present. A systematic analysis of these transcription factors is required to illustrate the gene regulation map of the *NDRG* family.

NDRG2 Functions as a Cell Stress Responsor

It is well known that a number of cell stress conditions induce the expression of human *NDRG1*. The first clue about NDRG1's reaction to cell stress conditions was that homocysteine, the sulfhydryl group-containing amino acid, up-regulates the expression of this gene [8], which has also been confirmed by a number of subsequent studies [10,57,64–67]. For instance, DNA-damaging agents induced *NDRG1* expression in a p53-dependent manner. NDRG1 protein demonstrated a cytoplasmic localization pattern with redistribution into the nucleus upon DNA damage [10]. Stein *et al* demonstrated that *NDRG1* acts as an indispensable contributor during p53-induced caspase activation and apoptosis [57]. The hypoxia and nickel reagent could induce human *NDRG1* expression in an HIF-1-dependent manner [65–67].

It is reasonable to predict the possibility that *NDRG2* works as a cell stress responding molecule as well. Wang *et al* investigated the involvement of *NDRG2* in hypoxia response and found that *NDRG2* expression was markedly up-regulated in several tumor cell lines exposed to hypoxic conditions or similar stresses at the mRNA and protein levels [34]. The expression of *NDRG2* was regulated by HIF-1 in tumor cells under hypoxia, and HIF-1 directly bound to HREs in the *NDRG2* promoter *in vivo*. Importantly, silencing or enforcing the expression of *NDRG2* may strongly inhibit or increase apoptosis.

Similar to NDRG1 protein upon DNA damage, NDRG2 can be translocated from the cytoplasm to the nucleus [34,68]. However, there is no explicit nuclear localization signal (NLS) sequence identified in NDRG2 protein. Although an NLS is the most common type of nuclear import element, other sequences are important for targeting certain proteins to the nucleus. For example, adenomatous polyposis coli and breast cancer 1 were recently shown to enter the nucleus via pathways that are independent of their NLS [69]. It could be speculated that NDRG2 may have its own motif that is directly or indirectly responsible for its nuclear translocation. Wang *et al* confirmed that the segment (101 to 178 amino acids) of NDRG2 is responsible for its nuclear translocation [34].

Although the exact physiological significance of NDRG2 in cell stress is unknown, the role of *NDRG2* and *NDRG1* in controlling cell cycle progress and apoptosis signal sensitivity has been well established [34,38,57]. Considering the phenomenon that many tumor suppressors belong to cell stress responding genes, it would be interesting to explore the detailed working mechanism of Ndrg proteins under stress. It may provide more clues of Ndrg protein as a therapeutic target of cancer.

NDRG2's Function in Nervous System

The *NDRG* gene family is expressed widely in the nervous system. Each member has its own distribution priority in different parts of the brain and cell-type specificity [32], suggesting this gene family may have a complicated role in nervous system. Disease-related gene structure or expression changes were noticed both in *NDRG1* and *NDRG2* [70–72]. Hereditary motor and sensory neuropathy-Lom (HMSNL), a severe autosomal recessive form of Charcot-Marie-Tooth disease, is a common cause of disability in adulthood. Kalaydjieva *et al* identified *NDRG1* as the causing gene of HMSNL [70]. A single point mutation, a premature-termination codon at position 148 was confirmed in HMSNL patients. Mutations in *NDRG1* accounted for 2.88% of the overall group of patients investigated and for 4.68% of the cases with demyelinating neuropathies [71]. HMSNL is a feature of Schwann cell dysfunction and concomitant early axonal involvement. The demyelination of peripheral nerves is one of the major pathologic changes in HMSNL patients, implicating *NDRG1* in Schwann cell signaling. The *NDRG1* deficiency mice model provided more evidences supporting *NDRG1*'s important function in Schwann cells differentiation [72]. Progressive demyelination in peripheral nerves is the major phenotype in *NDRG1* knockout mice, suggesting that this protein is essential for myelin sheath maintenance. Hirata *et al* observed *NDRG1* expression during the course of Wallerian degeneration and ensuing regeneration after injuring mouse sciatic nerves [73]. They found that *NDRG1* expression was maintained in the early stage of myelin degradation but markedly reduced at the end stage. Intriguingly, *NDRG1* expression increased at the stage of remyelination, with immunoreactivity stronger than that in intact nerves [73].

Because brain tissue is one of the most abundant tissues expressing *NDRG2* [5,7,55], the important function of this protein in the nervous system was undoubtedly expected and was confirmed by several studies [55,74,75]. Mitchelmore *et al* found that *NDRG2* is up-regulated at

both the RNA and protein levels in the brains of patients with Alzheimer's disease [74]. Expression of *NDRG2* in affected brains was found in cortical pyramidal neurons, senile plaques and cellular processes of dystrophic neurons. Overexpression of two splice variants encoding a long and short *NDRG2* isoform in hippocampal pyramidal neurons of transgenic mice resulted in localization of both isoforms to dendritic processes. Takahashi *et al* demonstrated that chronic treatment with a tricyclic antidepressant (i.e. imipramine) and a selective serotonin reuptake inhibitor (i. e. sertraline) reduced the expression of *NDRG2* mRNA and protein in the rat frontal cortex [75]. Repeated electroconvulsive treatment also significantly decreased *NDRG2* expression in this region of the brain, implying that NDRG2 may be associated with treatment-induced adaptive neural plasticity in the brain, a chronic target of antidepressant action [75]. As antidepressants may alleviate symptoms of depression by reversing the effects of glucocorticoids, increased *NDRG2* mRNA resulting from glucocorticoid treatment in astrocytes suggests that further study of NDRG2 regulation and function in glia could contribute to a better understanding of the pathogenesis and treatment of depression [56].

In addition, other studies reported the effects of *NDRG2* and *NDRG4* on neural cell differentiation [76,14]. NDRG2 promotes neurite outgrowth of NGF-differentiated PC12 cells [76]. The cells having decreased levels of the NDRG4 protein (antisense construct of rat *NDRG4* complementary DNA transfectants) extended shorter neurites than control cells in response to NGF or dibutyryl cAMP [14]. NGF-mediated activation of the transcription factor AP-1 was suppressed in the NDRG4 protein-diminished clones as compared with those in the control cells. NGF-induced phosphorylation of MEK and ERK was enhanced by NDRG4 protein [77]. Interestingly, *NDRG4* mRNA increased during hot water epilepsy in a rat model [78].

Taken together, the NDRG family is a group of proteins expressed widely in brain. Some family members are expressed differentially in diseases of the nervous system and regulated by the agents targeting neurons or astrocytes. The study of the physiological and pathological roles of these family members in the nervous system will be helpful for understanding the mechanism of related diseases.

The Other Function of NDRG2

NDRG2 has also been reported to play roles in other functions, such as insulin action [25], aldosterone-mediated epithelial sodium channel (ENaC) function [79,18], and dendritic cell (DC) differentiation [80,81].

NDRG2 protein is probably involved in insulin action [25]. It was directly phosphorylated by endogenous Akt upon stimulation of muscle cells with insulin. The Akt-mediated phosphorylation of NDRG2 at Thr348 is inhibited by PKCθ, which phosphorylated Ser332 of NDRG2. This crosstalk might represent one mechanism by which lipid-activated PKC interfere with insulin action [25].

Simultaneously, *NDRG2* has been identified as an early aldosterone-induced gene in rat kidneys [18]. Recently, Wielpütz *et al* found that *NDRG2* may affect ENaC function in *Xenopus laevis* oocytes and rat thyroid cells [79]. Co-expression of *NDRG2* significantly increased whole cell current in some, but not all, batches of oocytes tested. An *NDRG2*-induced increase in ENaC currents was accompanied by a similar increase in channel surface expression.

NDRG2's mRNA is nearly undetectable in the thymus, bone marrow, testes and peripheral blood leukocyte [5]. However, it was expressed in DCs derived from CD34+ progenitor cells and differentially regulated by maturation-inducing stimuli. The inhibition of DC differentiation by dexamethasone or vitamin D treatment decreased the expression of the *NDRG2* gene in DCs. In addition, gene expression was induced in a myelomonocytic leukemia cell line, which is capable of differentiating into DCs in cytokine-conditioned culture [80]. The expression of activated leukocyte cell adhesion molecule is down-regulated specifically in DC differentiated from *NDRG2* short interfering RNA-transfected monocytes. Furthermore, DCs differentiated from *NDRG2* short interfering RNA-transfected monocytes showed a reduced ability to induce T-cell proliferation [81]. It was also reported that the expression of *NDRG2* mRNA was induced by WT1 in CD34+ cells and leukemic U937 cells [54]. Therefore, NDRG2 is a cell differentiation regulator in some type of hematopoietic progenitors. Similar to NDRG2, NDRG1 may be a mast cell maturation-associated inducible protein [82,83].

Concluding Remark

The studies on the function and regulation of the *NDRG* family have provided plenty of information indicating the multiple roles of this gene family. The most interesting findings about *NDRG2* are as follows: (1) its expression negatively correlates to cancer progression and positively correlates to cell differentiation; (2) it is involved in cell stress response; (3) its expression changes in nervous system diseases; and (4) it may be an ENaC regulator. Future studies will need to evaluate *NDRG2*'s potential as

a biological marker or a therapeutic target of cancer. To elucidate the exact function and regulation mechanism of *NDRG2*, research of other *NDRG* family members will provide more information on the network controlled by Myc under normal and disease conditions.

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