

# N-myc Downstream Regulated Gene (NDRG): Role in Cancer Metastasis Suppression and as Drug Target in Cancer Therapeutics

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## Abstract

N-myc downstream regulated gene (NDRG) is, a ubiquitously expressed, a family of cytosolic proteins consists of four members, NDRG1-4. It plays an important role in cancer such as aberrant expression, tumor suppressive, metastatic suppressive and oncogenic functions. In this review, we comprehensively present the expression as well as the clinical and pathological significance of NDRG in human cancers. NDRG1 one of NDRG family which is a ubiquitously expressed protein localized in different tissues of the body, especially breast. It may act as a central regulator of multiple signaling pathways that modulate tumor progression. NDRG2 is expressed in brain, gastric, pancreatic and prostate cells. NDRG3 is highly expressed in testis, prostate, muscle and ovary. Whereas, NDRG4 is highly expressed in brain, heart and colorectal cells. NDRG is down-regulated and associated with the incidence, progression and prognosis of diverse cancers. It is associated with tumor incidence, progression, and metastasis. NDRG has low expression in cancer patients, whereas, inducement of NDRG activity has metastatic suppression effect and also increased in apoptotic effect through increased p53 activity. Metastasis is the spread of a cancer from one organ or part of the body to another not directly connected with it. NDRG is negatively correlated with tumor progression in multiple neoplasms, being a promising new target for cancer treatment. Hence, a drug that has a capacity to enhance an activity of NDRG has a potential to be considered as anti-metastatic agent.

**Keywords:** NDRG; Cancer; Metastasis; Drug target; Cancer therapeutics

## Introduction

The N-myc downstream regulated gene (NDRG) is a protein family consists of 4 members, NDRG1, NDRG 2, NDRG 3, and NDRG 4, which share 57-65% amino acid identity. Human NDRG1-4 is located on chromosomes 8q24.3, 14q11.2, 20q11.21-11.23, and 16q21-q22.1, respectively. Different names have been designated to the different family members, and each gene is transcribed into multiple isoforms with distinct mRNAs. These proteins are well conserved through evolution and NDRG1 is the first member to be discovered and responsible for the family name, because its expression is repressed by the proto-oncogenes MYCN and MYC [1-3].

NDRG1, also known as Drg1, Cap43, Rit42, RTP and PROXY-1, is a ubiquitously expressed, predominantly cytosolic protein. It was identified as a gene mutated in hereditary motor and sensory neuropathy-LOM (HMSNL; CMT4D) and mapped to human chromosome 8q24. Depending on the tissue type the NDRG1 protein is localized in the cytoplasm, nucleus, mitochondrion or membranes. The expression of NDRG1 may be altered by several factors such as hypoxia, heavy metals, DNA damage, hormones, oncogene, and tumor-suppressor genes [2,4-6]. Even though, the cellular and molecular function of these protein family members has not been clearly elucidated, all are characterized by hydrolase-fold motif [1-3].

Cap43 has been identified as a nickel- and calcium-induced gene, and is also known as NDRG1, Drg-1 and rit42. It is also reported that overexpression of Cap43 suppresses metastasis of some malignancies. This is one of the four closely related genes (NDRG1-4), the expression of which is down-regulated by c-myc or the N-myc/Max complex. Cap43 is also identical to the homocysteine-inducible gene, reduced in tumor cells (RTP/rit42; ref. 6), and to the differentiation-related gene-1 (Drg-1; ref. 7) [7,8].

The development of metastatic disease involves an orderly sequence

of multiple steps enabling tumor cells to migrate from the primary tumor and colonize at secondary locations in a cellular reprogramming of complex process. Dissemination to distant organs from the primary site is a complex process that involves multiple steps. The metastatic sites have a heterogeneous characteristic suggests that the cells establishing metastases have the ability to survive, self-renew, differentiate and modify [9].

## Role, expression and function of NDRG1 in cancer and its treatment

NDRG1 is a differentiation-related gene with putative metastasis suppressor activity in numerous cancers including breast, colon, cervix, ovaries, prostate, gastric, lung and pancreatic cancer. Its expression is decreased in cancer and metastatic cells when compared to normal cells [1-3,10]. Whereas, the ectopic overexpression of NDRG1 transcript has clinical significance and is correlates with poor patient survival, correlates with tumor differentiation grade and vascular invasion and also associated with indicators of poor prognosis in esophageal squamous cell carcinoma. NDRG1 is also positively linked to recognized markers of metastasis, angiogenesis and apoptotic evasion [2,4,11,12].

The NDRG1 gene encodes a growth related protein, and its transcription can be induced in response to stress. The p53 gene is

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implicated in regulation of the cell cycle, apoptosis, and the onset of cellular senescence. Induced p53 inhibits cancer cell proliferation by up-regulating NDRG1 expression following polyamine depletion. NDRG1 is one of the direct mediators of induced p53 following polyamine depletion and that p53-dependent NDRG1 expression plays a critical role in the negative control of cancer cell proliferation [2,11].

NDRG1 is an iron regulated gene that is markedly up-regulated by cellular iron-depletion using novel antitumor agents. NDRG1 up-regulation in cancer leads to a significant reduction in primary tumor growth, angiogenesis, and metastasis. Moreover, NDRG1 is positively correlated with an increased differentiation of cancer, as well as pathological stage, histological grading, and reduced invasion (Table 1) [1-3,13,14]. 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 [2,15].

Metastasis suppressor genes inhibit metastasis but do not affect the growth of primary tumors. It participates in the regulation of multiple steps in the metastatic process, including cancer cell invasion, survival in the bloodstream, and survival at the secondary site [16]. The low expression of metastasis suppressor genes in highly metastatic cancers is dedicated to the epigenetic control and in some cases post-translational regulation [17-19].

NDRG1 is a potent metastasis suppressor that has been demonstrated to inhibit the transforming growth factor b (TGF-b)-induced EMT by maintaining the cell-membrane localization of E-cadherin and b-catenin in cancer cells. E-cadherin is a tumor suppressor which is highly expressed in epithelial cells and plays crucial roles in cell-cell adhesion. The studies revealed that there is an association in expression of E-cadherin and NDRG in cancers such as prostate carcinoma. Down-regulation of E-cadherin expression is the hallmark of the epithelial-to-mesenchymal transition (EMT) process, the mechanism by which immotile epithelial cells convert to the motile mesenchymal phenotype [2,14,19,20]. Loss of E-cadherin expression or function is associated with cancer cell invasion and metastasis. Recently, it has been shown that promoter methylation of the NDRG1 gene was associated with reduced NDRG1 expression but not with histone modification in gastric cancer cells and tissue samples [14,19,21].

Thus, it seems that epigenetic modification of NDRG1 may vary among different cells and tissues. Some drugs such as Valproic acid and iron chelators has a potential to up-regulate NDRG1 in highly metastatic cancer cells but not in non-metastatic cancer cells and markedly slows tumor growth and acts as a potent metastasis suppressor [11,19,22,23]. Furthermore, up-regulation of NDRG1 by iron chelators inhibits the TGF-induced EMT [14,19,24].

Name	Alias	Chromosomal Location	Protein Length (aa)	Type of cancer	Expression	Function
NDRG1	CAP43, DRG1, RTP, NDR1, PROXY1, RIT42, TDD5, TARG1, CMT4D, NMSL, GC4, HMSNL	8q24	394	Breast	Expression is reduced in breast tumor cells, particularly in patients with lymph node or bone metastasis. Expression is associated with good prognosis. Molecular indicator of the therapeutic efficacy of anti-estrogenic agents.	Over-expression suppressed invasion <i>in vitro</i>
				Prostate	Expression is reduced in patients with lymph node or bone metastasis compared with those with localized prostate cancer. Expression is inversely correlated with Gleason grading and overall survival.	Over-expression reduced <i>in vitro</i> invasion and <i>in vivo</i> lung metastasis
				Colorectal	Expression is decreased in adenomas and adenocarcinomas and metastatic colon cancer. High expression is associated with resistance to Irinotecan. Expression is associated with good prognosis.	Over-expression inhibits <i>in vitro</i> invasion and <i>in vivo</i> liver metastasis and induced differentiation
				Esophageal (squamous)	Expression is lower in tumors of more advanced pathological stage (0-I vs. II-IV) and local tumor invasion (T1-2 vs.T3-4). Lower expression is associated with a shorter survival after surgery.	
				Brain (glioma)	Expression is lower in cancer and enhanced from grade IV to grade I glioma. High expression is associated with better survival.	
				Pancreatic	Expression is associated with good prognosis.	Over-expression decreased invasion, tumor growth, and angiogenesis
				Liver	High expression in cancer is associated with vascular invasion, metastasis, and shorter overall survival.	Silencing reduced proliferation, invasion, and apoptosis <i>in vitro</i> , and inhibited tumor growth <i>in vivo</i>
				Cutaneous squamous cell	Expression is increased in skin cancer compared to normal tissue	
				Oral squamous Cell	Expression was higher in oral squamous cell carcinoma compared to normal tissue. Higher expression is associated with poor differentiation.	
				Cervical	Inverse correlation with the overall survival. Expression was enhanced in invasive cervical cancer compared to carcinoma <i>in situ</i>	
Renal	Expression is higher in cancerous regions than in the non-cancer counterpart					

**Table 1:** Molecular features, expression and function of the human NDRG1 family members in cancer [1-3,10].

Significantly, NDRG1 up-regulation decreased tumor growth rate, and its expression was increased by DNA-damaging agents such as Act D. NDRG1 mRNA is up-regulated by chelators such as iron chelators but not the DNA-damaging agents. Up-regulation of NDRG1 mRNA and protein levels after iron chelation is rapid. Up-regulation of NDRG1 after iron chelation occurs by HIF-1 dependent and independent mechanisms [2,19,22].

Due its negative correlation with tumor progression in multiple neoplasms, NDRG1, is being a promising new target for cancer treatment. Primarily, NDRG1 is a cytoplasmic protein expressed mostly in epithelial cells but its cellular localization depends upon the cell type. For intestinal and lactating breast epithelial cells, NDRG1 is associated with the plasma membrane; for prostate epithelial cells, NDRG1 is mainly localized to the nucleus; whereas for kidney proximal tubule cells, NDRG1 is associated with the mitochondrial inner membrane. Mechanistic studies at the molecular level revealed that NDRG1 can regulate key signaling pathways involved in oncogenesis. Consequently, NDRG1 could interrupt many metastasis-associated biological functions, including the EMT, migration and invasion. Therefore, NDRG1 may act as a central regulator of multiple signaling pathways that modulate tumor progression [25].

### **Role, expression and function of NDRG2 in cancer and its treatment**

Human N-Myc downstream-regulated gene 2 (NDRG2), located at chromosome 14q11.2, has been reported to be down-regulated and associated with the progression and prognosis of diverse cancers. Collectively, previous studies suggest that NDRG2 functions as a candidate tumor-suppressor and cell stress-related gene. NDRG2 protein is a tumor suppressor that inhibits cancer growth, metastasis and invasion. It is associated with tumor incidence, progression, and metastasis. Thus, up-regulation of NDRG2 protein might act as a promising therapeutic strategy for malignant tumors [25-30]. In addition, NDRG2 is expressed in astrocytes, and may be involved in the modulation of gliocyte function in the central nervous system [31].

The aberrant methylation of NDRG2 may be mainly responsible for its down regulation in gastric cancer, and may play an important role in the metastasis of gastric cancer [27]. The study demonstrates that NDRG2 overexpression can inhibit tumor growth and invasion, furthermore, it can decrease bone destruction caused by prostate cancer bone metastasis [32]. NDRG2 may be involved in the carcinogenesis and progression of prostatic carcinoma. Moreover, adenovirus-mediated NDRG2 can suppress the proliferation of prostatic cancer cells significantly both *in vitro* and *in vivo*. These results indicate that NDRG2 may become a new target gene for prostatic carcinoma diagnosis and therapy [33]. The study demonstrated that the expression of NDRG2 genes is significantly altered in glioblastomas [34]. NDRG2 can regulate the function of NF- $\kappa$ B signaling pathway through the deubiquitylating enzyme of Zc3h12d [35]. The study demonstrated that miR-301a/b-NDRG2 might be an important axis modulating autophagy and viability of prostate cancer cells under hypoxia [36].

The study suggested that overexpression of NDRG2 increases iodine uptake and inhibits thyroid carcinoma cell growth *in situ* and *in vivo*. Thus, NDRG2 is a critical molecule in the regulation of medullary thyroid carcinoma biological behavior and a potential promoter in radioactive iodine therapy [37]. In addition, its overexpression reduces matrix metalloproteinase-2 and -9 activities and cell invasion of A549 lung cancer cell line *in vitro*. Thus, it is a new anti-invasion factor in lung cancer that inhibits matrix metalloproteinases activities [38]. The

study also demonstrated that overexpression of NDRG2 potentially inhibits osteoclast differentiation and plays a role in modulating the signal transduction pathway responsible for osteoclastogenesis [39]. The NDRG2 has a role in protecting ischemic-hypoxic injury and provide novel potential targets for future potent clinical therapies on cerebral ischemia injury [31]. NDRG2 overexpression increases myoblast proliferation and caspase 3/7 activities without increasing overall differentiation. Furthermore, NDRG2 attenuates H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and specific serine and threonine amino acid residues appear to contribute to its function in muscle cells [40]. It also inhibits proliferation and invasive potential of colorectal cancer SW48 cells, which likely occurs via suppression of metalloproteinase 9 activity [41].

The reduction of intercellular adhesion molecule 1 (ICAM1) expression by NDRG2 in breast cancer cells decreases osteoclast differentiation, and demonstrate that excessive bone resorption could be inhibited via ICAM1 down-regulation by NDRG2 expression [42]. The decreased expression of NDRG2 or the increased expression of CD24 is an important feature of lung adenocarcinoma [43]. The studies suggested that down regulation of NDRG2 may play an important role in advanced hepatoblastomas and pancreatic cancer. The suppressed expression of NDRG2 correlates with poor prognosis in hepatoblastomas, breast cancer, lung adenocarcinoma and pancreatic cancer [42-45].

On the other hand, the study suggested that NDRG2 promoted secreted miR-375 in microvesicles shed from M1 microglia, which induced neuron damage. The suppression of NDRG2 and secreted miR-375 in MVs shed from M1 microglia may be potential targets for alleviation of neuron damage. Thus, NDRG2 may be a potential marker in the astrocytoma prognosis [46-48]. When NDRG2 is coexpressed with GluK2 and SGK1, the stimulating effect of SGK1 on GluK2 is suppressed both in heterologous expression and in astrocytes. Here, the study revealed a new negative feedback mechanism, whereby GluK2 stimulation by SGK1 is regulated by parallel phosphorylation of NDRG2. This regulation of GluK2 by SGK1 and NDRG2 in astrocytes may play an important role in gliotransmission, modulation of gene expression and regulation of exocytosis of tPA [49]. The study evidenced that the aberrant expression of NDRG2 may contribute to the malignant progression of prostate cancer. More importantly, the downregulation of NDRG2 may be efficient prognostic indicators for prostate cancer [50] (Table 2).

### **Role, expression and function of NDRG3 in cancer and its treatment**

The NDRG3 cDNA is 2588 base pair in length, encoding a 363 amino acid polypeptide highly related to mouse Ndr3 protein. NDRG3 is highly expressed in testis, prostate and ovary [51]. NDRG3 is also relatively ubiquitously expressed in heart, muscle, and brain [52]. The NDRG3 mRNA was localized to the outer layers of seminiferous epithelium, indicating that it may play a role in spermatogenesis [51]. NDRG3 is a tumor promoter, the overexpression of which may contribute to the malignant phenotype of tumors. NDRG3 is an androgen regulated and prostate enriched gene that promotes *in vitro* and *in vivo* prostate cancer cell growth [53].

The study demonstrated that a lactate-dependent signaling pathway in hypoxia, mediated by the oxygen- and lactate-regulated protein NDRG3. Oxygen negatively regulates NDRG3 expression at the protein level via the PHD2/VHL system, whereas lactate, produced in excess under prolonged hypoxia, blocks its proteasomal degradation by binding to NDRG3. In addition, the stabilized NDRG3 protein promotes

Name	Alias	Chromosomal Location	Protein Length (aa)	Type of cancer	Expression	Function
NDRG2	DKFZp781G1938, FLJ25522, KIAA1248, SYLD	4q11.1-11.2	371	Glioblastoma	Reduced expression in high-grade glioblastomas expression compared to normal and low-grade glioblastoma	Overexpression reduced proliferation <i>in vitro</i>
				Colon	Expression is down-regulated in cancer and adenomas compared to normal tissue. Expression is lower in invasive cancer	Silencing increased proliferation <i>in vitro</i>
				Pancreatic	Expression is reduced in cancer compared to normal tissue	
				Gastric	Expression is decreased in cancer compared to normal tissue. Survival is lower in NDRG2-negative patients.	Silencing increased proliferation <i>in vitro</i>
				Liver	Expression was decreased in cancer compared to normal tissue. Decreased expression in cancer is associated with aggressive behavior.	Overexpression suppressed invasion and migration <i>in vitro</i> and reduced metastasis <i>in vivo</i>
				Renal (Clear cell)	Expression is down-regulated in cancer compared to normal tissue	
				Meningioma	Expression is lower in high-grade cancer lower in grade III meningioma compared to grade I meningioma.	

Table 2: Molecular features, expression and function of the human NDRG2 family members in cancer [1-3,10].

Name	Alias	Chromosomal Location	Protein Length (aa)	Type of cancer	Expression	Function
NDRG3	RP3-460J8.1, FLJ13556	20q11.21-q11.23	375	Prostate	Expressed in both epithelial prostate cancer cells and prostatic stromal cells.	Over-expression increased proliferation and migration <i>in vitro</i> and promotes proliferation <i>in vivo</i>

Table 3: Molecular features, expression and function of the human NDRG3 family members in cancer [1-3,10].

angiogenesis and cell growth under hypoxia by activating the Raf-ERK pathway. Inhibiting cellular lactate production abolishes NDRG3-mediated hypoxia responses. The NDRG3-Raf-ERK axis therefore provides the genetic basis for lactate-induced hypoxia signaling, which can be exploited for the development of therapies targeting hypoxia-induced diseases in addition to advancing our understanding of the normal physiology of hypoxia responses [54,55].

The study evidenced that the aberrant expression of NDRG3 may contribute to the malignant progression of prostate cancer. More importantly, the upregulation of NDRG3 may be efficient prognostic indicators for prostate cancer [50]. NDRG3 was up-regulated in HepG2.2.15 and was identified as a target gene of miR-122. An inverse correlation between the expression of miR-122 and the NDRG3 protein was noted in hepatitis B virus-related hepatocellular carcinoma specimens. NDRG3 represent key diagnostic markers and potential therapeutic targets for HBV-related HCC [56] (Table 3).

### Role, expression and function of NDRG4 in cancer and its treatment

NDRG4 is a member of the NDRG family, which is highly expressed in brain and heart. In heart, it has been reported to modulate cardiac development and QT interval duration. It is also expressed in smooth muscle cells of the stomach, macrophages of the spleen and neurons [57-60]. NDRG4 is a novel candidate tumor suppressor and can inhibit PI3K/AKT signal which is related with energy balance and related carcinogenesis [61,62]. The study demonstrated that NDRG4 deficient mice had spatial learning deficits and vulnerabilities to cerebral ischemia [60,63].

The increased NDRG4 expression and its upregulation aggravate myocardial ischemia/reperfusion injury by inhibiting the activation of the reperfusion injury salvage kinase pathway, thereby

identifying NDRG4 as a potential therapeutic target in ischemia/reperfusion injury [59]. The study evidence that NDRG4 level in colorectal cancer could effectively stratify the prognostic value of obesity, which would better the understanding of the prognostic and biomarker role of obesity in colorectal cancer [61,62].

NDRG4 protein is overexpressed in aggressive meningioma. NDRG4 down regulation also decreased cellular invasion and migration [64]. NDRG4 is associated with p53 and regulator of apoptosis in malignant meningioma cells [65]. NDRG4 is involved in modulating cell proliferation, invasion, migration and angiogenesis in meningioma, and may play a valuable role as a molecular target in its treatment [64]. The study indicated that NDRG4 protein expression was decreased in colorectal cancer. It may play its tumor suppressive role in carcinogenesis and progression through attenuation of PI3K-AKT activity. Therefore, high risk colorectal cancer patients could be better identified based on the combination of NDRG4 and PI3K-AKT activity. It is a predictor of overall survival of colorectal cancer patients [66,67].

NDRG4 has roles in supporting the activation of ERK and its target proteins needed for neuronal differentiation, neurite formation and in reducing the activation of Elk-1 implicated in cell growth. Its expression is significantly altered and downregulated in human gliomas. The glioma patients with lower NDRG4 expression have a poor prognosis [34,68-73]. NDRG4 is critical for regulation of epicardial cell directional movement, as disruption of this interaction randomizes migratory patterns [74,75] (Table 4).

### Conclusion

NDRG has four protein families from 1 to 4 distributed in different tissues of the body. It's over expression and down expression has an association with metastasis and carcinogenesis. NDRG is a metastasis

Name	Alias	chromosomal Location	Protein Length (aa)	Type of cancer	Expression	Function
NDRG4	DKFZp686l1615, FLJ30586, FLJ42011, KIAA1180, MGC19632, BDM1, SMAP8	16q21-q22.1	352	Colorectal	Expression is diminished in cancer compared to normal cells.	Overexpression reduced proliferation and invasion <i>in vitro</i>
				Gliomas	Expression is increased in glioblastoma cells compared to normal human cortex.	Knockdown causes G1 cell cycle arrest followed by apoptosis

**Table 4:** Molecular features, expression and function of the human NDRG4 family members in cancer [1-3,10].

suppressor gene and associated with different cancer types. NDRG1 is associated with breast, prostate, cervical, liver, pancreatic, glioma, colorectal, renal, oral squamous cell, cutaneous squamous cell and esophageal squamous cell cancers. NDRG2 is associated with glioblastoma, colon, gastric, pancreatic, liver, meningioma and renal cancer. NDRG3 is associated with prostate cancer. NDRG4 is associated with colorectal cancer and gliomas. NDRG has a clinical significance as a biomarker for diagnosis of different cancer types. In addition, it was considered as a potential target for anticancer drug discovery development and cancer treatment.

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