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Review Article
N-myc Downstream Regulated Gene1 (NDRG1) in Cancer Metastasis and 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 and important role in cancer such as aberrant expression, tumor suppressive, metastatic suppressive and oncogenic functions. To review the role of N-myc downstream regulated gene in metastasis suppression and its contribution as a target in drug discovery and development as well as cancer therapeutics. Reports and publications were found in the peer-reviewed and grey literature through academic search engines and web searches. The studies were reviewed and explored in more depth. Metastasis is the spread of a cancer from one organ or part of the body to another not directly connected with it. The NDRG1 one of NDRG family, which is a ubiquitously expressed protein localized in different tissues of the body. The NDRG1 may act as a central regulator of multiple signaling pathways that modulate tumor progression. It 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. The NDRG1 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 NDRG1 has a potential to be considered as anti-metastatic agent.

Key words: NDRG, cancer, metastasis, metastatic suppression, drug target, drug discovery, drug development, cancer therapeutics

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The N-myc Downstream Regulated Gene (NDRG) is a protein family consists of 4 members, NDRG1, NDRG2, NDRG3 and NDRG4, 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]. The 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[2,4,5]. Even though, the cellular and molecular function of these protein family members has not been clearly elucidated, all are characterized by hydrolase-fold motif (Table 1)[1-5].

The Cap43 has been identified as a nickel and calcium-induced gene and is also known as NNDRG1, 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. The Cap43 is also identical to the homocysteine-inducible gene, reduced in tumor cells (RTP/Rit42)[6] and to the differentiation-related gene-1 (DRG1)[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].

EXPRESSION AND FUNCTION OF NDRG IN CANCER

The NDRG family may play an important role in cancer such as aberrant expression, tumor suppressive and oncogenic functions affecting carcinogenesis such as cell proliferation, differentiation, migration, invasion and stress response have been reported for several of the NDRG proteins. In addition, mutations in these genes are associated with diverse neurological and electrophysiological syndromes. The NDRG1 (mRNA and protein) expression is decreased in cancer and metastatic cells when compared to normal cells. The NDRG1 is regarded as a metastasis suppressor gene in cancers of the pancreas, colon, breast, cervix, ovaries and prostate. This has been described for colon, prostate, breast, esophageal squamous cancer and glioma (Table 2)[1,3,10].

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

Table 1: Molecular features of the human NDRG family members

Name	Alias	Chromosomal location	RefSeq ID	UniProt ID	Isoform (UniProt)	Protein length (aa)
NDRG1	CAP43, DRG1, RTP, NDR1, PROXY1, RIT42, TDD5, TARG1, CMT4D, NMSL, GC4 and HMSNL	8q24	NM_006096	Q9259	-	394
NDRG2	DKFZp781G1938, FLJ25522, KIAA1248 and SYLD	14q11.1–11.2	NM_201535, NM_201539, NM_201540, NM_201536, NM_016250, NM_201538 and NM_201541	Q9UN36	1	371
					2	357
					3	360
					4	328
					5	341
NDRG3	RP3-460J8.1 and FLJ13556	20q11.21–q11.23	NM_032013 NM_022477	Q9UGV2	1	375
					2	363
					3	286
NDRG4	DKFZp686I1615, FLJ30586, FLJ42011, KIAA1180, MGC19632, BDM1 and SMAP8	16q21–q22.1	NM_020465 and NM_022910	Q9ULP	1	352
					2	339
					3	371
					4	339
					5	357
					6	391

Table 2: Expression and function of the NDRG family members in cancer

Name	Type of cancer	Expression	Function
NDRG1	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 *in vitro*
	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 *in vitro* invasion and *in vivo* 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 *in vitro* invasion and *in vivo* 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 *in vitro* and inhibited tumor growth *in vivo*
	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 *in situ*	
	Renal	Expression is higher in cancerous regions than in the non-cancer counterpart	
NDRG2	Glioma blastoma	Reduced expression in high-grade glioblastomas expression compared to normal and low-grade glioblastoma	Overexpression reduced proliferation *in vitro*
	Colon	Expression is down-regulated in cancer and adenomas compared to normal tissue. Expression is lower in invasive cancer	Silencing increased proliferation *in vitro*
	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 *in vitro*
	Liver	Expression was decreased in cancer compared to normal tissue. Decreased expression in cancer is associated with aggressive behavior	Overexpression suppressed invasion and migration *in vitro* and reduced metastasis *in vivo*
	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	
NDRG3	Prostate	Expressed in both epithelial prostate cancer cells and prostatic stromal cells	Over-expression increased proliferation and migration *in vitro* and promotes proliferation *in vivo*
NDRG4	Colorectal	Expression is diminished in cancer compared to normal cells	Overexpression reduced proliferation and invasion *in vitro*
	Gliomas	Expression is increased in glioblastoma cells compared to normal human cortex	Knockdown causes G1 cell cycle arrest followed by apoptosis

following polyamine depletion. The 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].

ROLE OF NDRG1 IN CANCER AND ITS TREATMENT

The NDRG1 is a differentiation-related gene with putative metastasis suppressor activity and its overexpression has clinical significance. The ectopic overexpression of NDRG1 transcript is correlates with poor patient survival, correlates with tumor differentiation grade and vascular invasion and also associated with indicators of poor prognosis in cancer. It also positively linked to recognized markers of metastasis, angiogenesis and apoptotic evasion[2-4,12].

The metastasis suppressor gene, NDRG1 is negatively correlated with tumor progression in multiple neoplasms. Meanwhile, NDRG1 is an iron regulated gene that is markedly

up-regulated by cellular iron-depletion using novel antitumor agents. The 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[2,3,13,14]. The MYC exerts its biological functions mainly through transcriptional regulation of its target genes, which are involved in cell's interaction and communication with their external environment[2,15].

Metastasis suppressor genes inhibit metastasis but do not affect the growth of primary tumors. The expression or function of metastasis suppressor genes is lost primarily in many metastatic cancers. Interestingly, restoration of metastasis suppressor gene expression could inhibit cancer metastasis. Metastasis suppressor proteins participate 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].

The NDRG1 is a metastasis suppressor gene and its implication in cancer progression and metastasis has been extensively studied. The anti-metastatic function of NDRG1 as a metastasis suppressor protein has been identified in multiple cancers including breast, colon, prostate and gastric cancers. E-cadherin is a tumor suppressor which is highly expressed in epithelial cells and plays crucial roles in cell-cell adhesion. 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[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].

The NDRG1 is a potent metastasis suppressor that has been demonstrated to inhibit the Transforming Growth Factor-β (TGF-β)-induced EMT by maintaining the cell-membrane localization of E-cadherin and β-catenin in cancer cells. The mechanism of inhibiting β-catenin phosphorylation involves the NDRG1-mediated up-regulation of the GSK3β-binding protein FRAT1, which prevents the association of GSK3β with the axin1–APC–CK1 destruction complex and the subsequent phosphorylation of β-catenin. Additionally, NDRG1 is shown to modulate the WNT–β-catenin pathway by inhibiting the nuclear translocation of β-catenin. This is mediated through an NDRG1-dependent reduction in the nuclear localization of p21- activated kinase 4 (PAK4), which is known to act as a transporter for b-catenin nuclear translocation[2,19].

Incubation of cells with chelators markedly increased NDRG1 mRNA and protein expression, but this was not found with their Fe complexes or when the Fe-binding site had been inactivated. Increased NDRG1 expression following Fe chelation was related to the permeability and antiproliferative activity of chelators and could be reversed by Fe repletion. Moreover, NDRG1 up-regulation after chelation occurred at the transcriptional level and was mediated by Hypoxia Inducible Factor-1 (HIF-1) dependent and independent mechanisms, which suggests NDRG1 is a novel link between Fe metabolism and the control of proliferation. The NDRG1 has been shown to be down-regulated in tumor cell lines as well as in breast, prostate and invasive colorectal cancers. Significantly, NDRG1 up-regulation decreased tumor growth rate and its expression was increased by DNA-damaging agents such as Act D. The NDRG1 mRNA is up-regulated by 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].

The metastasis suppressor, NDRG1, is negatively correlated with tumor progression in multiple neoplasms, 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, including: (i) The NF-κB pathway, (ii) The phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signaling network, (iii) Ras/Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, (iv) Transforming growth factor-β (TGF-β) network and (v) The Wnt pathway. 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].

CONCLUSION

The NDRG has four protein families from 1-4 distributed in different tissues of the body. It's over expression and under expression has an association with metastasis and carcinogenesis. The NDRG1 is one of the families which are a metastasis suppressor gene in different cancer types. Therefore, it was considered as a potential target for anticancer drug discovery development and cancer treatment.

SIGNIFICANCE STATEMENT

This manuscript compiled in detail review of the description of NDRG and its families and contribution of NDRGs in metastasis suppression; importance of NDRG as a target in drug discovery and development as well as cancer therapy.

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