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Review

Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy

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ABSTRACT

N-myc down-regulated gene 1 (NDRG1) is a known metastasis suppressor in multiple cancers, being also involved in embryogenesis and development, cell growth and differentiation, lipid biosynthesis and myelination, stress responses and immunity. In addition to its primary role as a metastasis suppressor, NDRG1 can also influence other stages of carcinogenesis, namely angiogenesis and primary tumour growth. NDRG1 is regulated by multiple effectors in normal and neoplastic cells, including N-myc, histone acetylation, hypoxia, cellular iron levels and intracellular calcium. Further, studies have found that NDRG1 is up-regulated in neoplastic cells after treatment with novel iron chelators, which are a promising therapy for effective cancer management. Although the pathways by which NDRG1 exerts its functions in cancers have been documented, the relationship between the molecular structure of this protein and its functions remains unclear. In fact, recent studies suggest that, in certain cancers, NDRG1 is post-translationally modified, possibly by the activity of endogenous trypsins, leading to a subsequent alteration in its metastasis suppressor activity. This review describes the role of this important metastasis suppressor and discusses interesting unresolved issues regarding this protein.

Contents

Abbreviations: Akt, protein kinase B; CPT-11, irinotecan; CMT4D, Charcot–Marie–Tooth disease, type 4D; DFO, deferoxamine (or desferrioxamine B); DpC, di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone; DpT, di-2-pyridylketone thiosemicarbazone; Dp44mT, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone; ECM, extracellular matrix; ERG-1, v-ets avian erythroblastosis virus E26 oncogene homologue; EGR1, early growth factor-1; ETS, v-ets avian erythroblastosis virus E26 oncogene homologue 2; HDL-C, high-density lipoprotein cholesterol; HIF-1α, hypoxia inducible factor-1α; HMSNL, hereditary motor and sensory neuropathy-Lom; Hsc70, 70-kDa heat shock cognate protein; HTE, human tracheal epithelial cell; HUVEC, human umbilical vein endothelial cell; IL, interleukin; LDL, low-density lipoprotein; LPC, lysophosphatidylcholine; MMP, matrix metalloproteinase; NDRG1, N-myc down-stream regulated gene 1; NF-κB, nuclear factor-κB; NIH, 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone; PAR-1, protease activated receptor; PKA, protein kinase A; PKA, protein kinase C; PrEC, prostate epithelial cell; pMLC2, phosphorylated myosin light chain 2; PTEN, phosphatase and tensin homologue deleted on chromosome 10; pVHL, von Hippel–Lindau protein; ROCK1, Rho-associated, coiled-coil containing protein kinase 1 (ROCK1); ROS, reactive oxygen species; SGK, serum- and glucocorticoid-induced kinase; TAT, tumour-associated trypsinogen; TAT2, tumour-associated trypsinogen-2; TCF/LEF, T-cell factor/lymphoid enhancer-binding factor; Thtpa, thiamine triphosphatase; VEGF, vascular endothelial growth factor

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1. Introduction

Cancer is a major public health issue, with one in four deaths in the United States attributable to the disease [1]. Cancers encompass multiple entities of complex and multifactorial diseases [2], each having its own specific aetiology and genomic, histological and clinical characteristics [3,4]. Despite recent advances, effective clinical management remains elusive due to intra-tumoural heterogeneity and therapeutic resistance [5–11]. Therefore, novel therapies and further investigation into the pathophysiology of cancer are essential [12,13].

Metastasis is the process by which cancer cells disseminate from a primary tumour site to a distal secondary tissue microenvironment [14] and therefore represents an important therapeutic target [15]. Metastasis markedly complicates clinical management and is a major cause of death due to cancer [16]. Growing evidence has underscored the involvement of oncogenes, tumour suppressor genes and metastasis suppressor genes in the development and progression of cancer [17–20]. One metastasis suppressor, namely N-myc down-stream regulated gene 1 (NDRG1), is of particular interest and plays an anti-oncogenic role in cancers of the brain [21], breast [22,23], colon and rectum [24–28], oesophagus [29], pancreas [30] and prostate [31]. Paradoxically, NDRG1 is highly expressed in cancers of the kidney [32], liver [33–35], mouth [36], skin [37] and uterine cervix [38,39], where it has been suggested to play a role in promoting tumourigenesis.

Human NDRG1, the inaugural member of the NDRG family, was first identified as a predominantly cytoplasmic protein encoded by the ubiquitously expressed *N-myc down-stream regulated gene 1* (*NDRG1*) [24,40]. The molecule has been referred to under a number of different designations, including reducing agent and tunicamycin-responsive protein (RTP) [40], differentiation-related gene (Drg)-1 [24], reduced in tumours, 42-kDa protein (Rit42) [41], Ca^{2+}-associated protein 43 (Cap43) [42] and protein regulated by oxygen (PROXY)-1 [43]. The current designation, "NDRG1", has been agreed upon by a consortium of NDRG1 researchers and is the currently accepted gene symbol assigned by the HUGO Gene Nomenclature Committee [44]. Orthologues of *NDRG1* have been identified in the mouse, as *Ndr1* [45] and *TDD5* [46], and the common sunflower (*Helianthus annuus L.*), as *sf21* [47]. Therefore, *NDRG1* appears to be highly conserved across a wide range of organisms, indicating its important function in the cell.

2. Molecular structure of NDRG1 and its interactions

NDRG1 is a member of the NDRG family [48], currently consisting of *NDRG1*, *NDRG2*, *NDRG3* and *NDRG4* [49,50]. Notably, phylogenetic analysis indicates that each NDRG family member forms a separate homology cluster across multiple species with possibly specific and functionally divergent roles relative to the other family members (Fig. 1A) [51]. This group of genes is subdivided into two subfamilies, one of which is composed of *NDRG1* and *NDRG3* and the other is composed of *NDRG2* and *NDRG4* [50]. Proteins encoded by this family share approximately 57–65% amino acid identities with each other (Fig. 1B, C) [49,50] and are characterised by an NDR protein domain, consisting of an esterase-/lipase-/thioesterase-active-site serine and an α/β hydrolase fold of approximately 220 amino acids [50].

Although NDRG proteins do not possess a catalytic triad consisting of nucleophile–acid–histidine residues that is required for function as a hydrolase [48,52], the proteins belong to the α/β hydrolase superfamily [48]. The various *NDRG* family member genes may have arisen from the convergent evolution for this specific molecular architecture, but with distinct conformations and functions [53]. In fact, analyses indicate that the *NDRG* genes arose from multiple duplication events during evolution [51].

NDRG1 is mapped to the long arm of chromosome 8 [54], specifically to the locus 8q24.2 [55] or, in agreement with the AceView database [56], 8q24.3 (Fig. 2A) [41]. Spanning 60,085-bp, *NDRG1* contains 16 exons and 15 introns, and it encodes a 2997-bp mRNA, 1182 bp of which are coding (Fig. 2B) [24,55]. *NDRG1* mRNA is translated into a deduced 42,835-Da, 394-amino acid protein with a predicted pI of 5.7 [24,40,42,49]. The chromosomal region in which *NDRG1* is located frequently undergoes genetic alteration in cancers, such as those of the prostate [57], and can be amplified in other tumours [44]. The proto-oncogene, *c-myc,* is also located in close proximity to *NDRG1*, at the locus 8q24.21 [58] and is also frequently amplified in tumours [59,60]. Interestingly, c-myc, like N-myc, can repress *NDRG1* transcription and is implicated in causing a more metastatic phenotype [44,61].

Human NDRG1 differs from other NDRG proteins by the inclusion of not one but three decapeptide tandem repeats [49], each of which consists of the residues GTRSRSHTSE (Fig. 2C) [40]. In addition, other NDRG proteins also lack the *C*-terminal two residues, Ser and Glu [62]. The *C*-terminal domain of NDRG1 is unique amongst the NDRG proteins, as it can act as a substrate for phosphorylation by serum- and glucocorticoid-induced kinase (SGK)-1, which primes NDRG1 for phosphorylation by glycogen synthase kinase (GSK)-3β [63,64]. Specifically, SGK-1 has been confirmed to phosphorylate NDRG1 at Thr^{328}, Ser^{330}, Thr^{346}, Thr^{356} and Thr^{366} [65,66], while GSK-3β subsequently phosphorylates NDRG1 at Ser^{342}, Ser^{352} and Ser^{362} [63]. Thus far, enhanced phosphorylation *via* increased SGK-1 activity has been associated with severe Alzheimer's disease, but the underlying mechanisms have not been elucidated [67]. Moreover, phosphorylation of NDRG1 is a reversible process that has been suggested to influence the localisation of NDRG1 [68,69]. Further, phosphorylation of NDRG1 is putatively associated with the multiple physiological roles of NDRG1, including cell differentiation [70], centromere function during mitosis [71] and partitioning of daughter cells during telophase [71].