Chapter 11

NDRG1 in prostate cancer biology and treatment

Chapter 11a

NDRG1 (N-myc downstream regulated gene 1) [gene card]

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Identity

Other names: CAP43; CMT4D; DRG-1; DRG1; GC4; HMSNL; NDR1; NMSL; PROXY1; RIT42; RTP; Rit42; TARG1; TDD5

HGNC (Hugo): NDRG1

Location: 8q24.22

DNA/RNA

NDRG1 consists of 60,085 basepairs, starting at basepair 134,318,596 and ending at basepair 134,378,680 from the p-terminus. It is a member of the NDRG family, consisting of NDRG1, NDRG2, NDRG3 and NDRG4 (of which three isoforms exist: NDRG-4B, NDRG-4Bvar and NDRG-4H), which are part of the alpha/beta hydrolase superfamily. The DNA of NDRG1 contains 16 exons (Fig. 1). It encodes a 3.0 kb mRNA with a coding region of 1.185 kb.

Protein

NDRG1 is a 43 kDa protein, composed of 394 amino acids, with an iso-electric point of 5.7 (Fig. 2). NDRG1 has an alpha/beta hydrolase-fold motif, however, the presence of hydrolytic catalytic activity is still questionable. NDRG1 has more than seven phosphorylation sites, among others a phosphopantetheine attachment site, protein kinase C, casein kinase II, tyrosine kinase, protein kinase A and calmodulin kinase II. NRDG1 is phosphorylated by protein kinase A and calmodulin kinase II, and is a physiological substrate of SGK1 and GSK-3β kinase, a kinase involved in cancer growth and progression. NDRG1 is relatively ubiquitously expressed in normal human cells, and especially highly expressed in prostate, brain, kidney, placenta, ovarian, testicular and intestinal cells. NDRG1 is mostly found in epithelial cells. In cells, NDRG1 is primarily a cytoplasmic protein. 47.8% of the NDRG1 is expressed in the cytosol, 26.1% in the nucleus (such as in prostate epithelial cells), and 8.7% in the mitochondria (such as in proximal tubule cells in the kidney). NDRG1 is also found in the adherens junctions. Additionally, in intestinal and lactating breast epithelia NDRG1 is located in the plasma membrane. NDRG1 can also be found in vacuoles, the peroxisome, early and recycling endosomes, and the cytoskeleton.

NDRG1 amino acid sequence is 53% homologous to NDRG2, 62% to NDRG3, 62% to NDRG4, and 94% homologous to the mouse analog, Ndr-1 (also known as TDD5). NDRG1 homologs have been found in Helianthus, Caenorhabditis, Xenopus and Drosophilia.

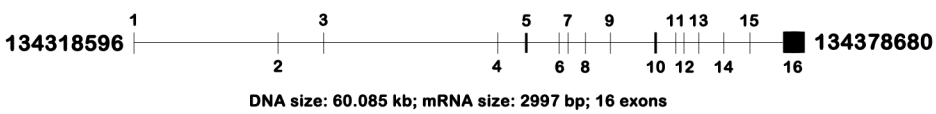

Figure 1. NDRG1 DNA

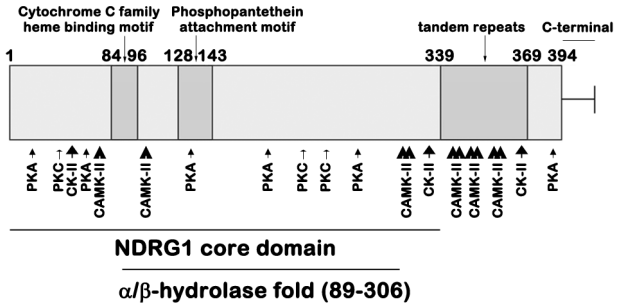

Figure 2. NDRG1 protein.

Function

NDRG1 is reported to be a metastasis suppressor gene which is downregulated in prostate, colon and breast cancers. It has been found to be a Rab4a effector protein that recruits to the recycling endosomes in the trans-Golgi network by binding to the lipid phosphotidylinositol 4-phosphate (PI4P), where it plays a role in the recycling of E-cadherin. NDRG1 also interacts with HSP70. NDRG1 co-localizes with APO A-I and A-II, and may be involved in lipid transport. The function of NDRG1 may be controlled at least in part by phosphorylation. It has also been identified as a stress response gene, upregulated by homocysteine and hypoxia. HIF-1-dependent and independent mechanisms have been implicated in NDRG1 induction. It is also controlled by AP-1 transcription factors. When exposed to stress, for example hypoxia, NDRG1 may play a cytoprotective role in normal healthy cells. There is evidence that NDRG1 is involved in the induction of differentiation. NDRG1 is downregulated under conditions of cell growth. NDRG1 expression peaks in the G1 and G2/M phases, and is lowest in the S phase. NDRG1 is also a microtubule-associated protein, which may play an important role in maintaining spindle structure during cell division. In Schwann cells, NDRG1 is essential for myelin sheath maintenance. Hence, NDRG1 is a multifunctional protein with roles that may be tissue- and/or cell-type specific.

Certain transcription factors such as myc, ERG1, HIF-1α bind to the NDRG1 promoter region and regulate its expression.

NDRG1 is upregulated during colon epithelial cell differentiation. It is regulated by hormones such as androgens and estradiol. Small molecules such as Nhydroxy- N'-phenol-octane-1,8-diotic acid diamide, calcium ionophores like BAPTA, metal ions such as Nickel and Cobalt, iron chelators and differentiating agents, such as retinoic acid, induce NDRG1 expression. Additionally, NDRG1 is induced during cellular DNA damage and endoplasmic reticulum stress.

Implications in solid cancers

NDRG1 has implications in prostate, breast, colon, renal, bladder, pancreatic and hepatic

cancer. Downregulation of NDRG1 in cancer worsens the prognosis of cancer. There is an inverse relationship in the levels of NDRG1 expression and the Gleason grade of the tumor in prostate cancer. A high PTEN (a tumor suppressor which positively regulates NDRG1) and NDRG1 expression improves survival rates in patients with breast and prostate cancer. In patients with colorectal cancer, the 2 year survival rate for patients with high NDRG1-expression was 82.4%, while for patients with a low NDRG1-expression it was only 69.6%. In pancreatic cancer patients, the median survival time for patients with high NDRG1-expression was 24.7 months, while the median survival time for patients with low NDRG1-expression was only 10.9 months. High expression of NDRG1 in colon tumors was found to correlate with increased resistance to irinotecan.

An inverse relationship exists between NDRG1 and the oncogenes N-myc and c-myc, suggesting that members of the myc family suppress expression of NDRG1. Experimental evidence exist that both N-myc and c-myc downregulate NDRG1 gene expression by directly binding to NDRG1 promoter.

NDRG1 is downregulated in colon, breast, prostate and pancreatic neoplasms, by c-myc and N-myc transcription factors. In cancer cells, NDRG1 expression is consistent through all phases in the cell cycle, instead of the biphasic expression in normal cells. PTEN expression is positively related to NDRG1 expression. NDRG1 is induced in cancer cells by histone deacetylase inhibitors and DNA methyl transferase inhibitors indicating that NDRG1 is regulated by chromatin modulation and DNA methylation.

Although NDRG1 has been reported to be downregulated in a variety of cancers, it has been shown to be upregulated in hepatic, pancreatic and kidney cancers. Induction of NDRG1 in these tumors is speculated to be in response to tumor stress or hypoxia, and NDRG1 is proposed as a marker for tumor hypoxia. However, in pancreatic cancer, cellular differentiation and not hypoxia was demonstrated to be the determining factor for NDRG1 expression. In renal cancer, induction of NDRG1 in the tumor tissue was restricted to infiltrating macrophages and not cancer cells.

NDRG1 is suggested to be an early target for p53. Loss of p53 expression in cancer is suggested to reduce NDRG1 expression. However, p53 knockout mice show expression of NDRG1, suggesting that there are other mechanisms regulating NDRG1 levels.

NDRG1 expression plays a role *in vitro* in primary tumor growth in prostate, breast, and bladder cancer: a higher expression of NDRG1 lowers the proliferation rates of these cancers. In pancreatic and bladder cancer cells, this reduction was proven *in vivo*: in pancreatic cells it was suggested that the reduced proliferation was caused by NDRG1 by modulating tumor stroma and angiogenesis. NDRG1 can recruit onto the recycling endosome in the trans-Golgi network by binding to phosphotidylinositol 4-phosphate. There, NDRG1 may be involved in the transport of various cargo back to the cells' surface. At the molecular level, NDRG1 may stabilize the E-cadherin molecule by recycling it back to the cells' surface, thereby preventing tumor invasion.

Implications in hereditary motor and sensory neuropathy-Lom (HMSNL) / Charcot-Marie-Tooth disease (CMT 4D)

HMSNL/CMT 4D is caused by the Gypsy founder mutation in NDRG1, homozygote R148X, also called homozygote C564t. In patients with CMT disease, apart from the R148X mutation, another disease-causing mutation was identified, namely IVS8-1G>A (g.2290787G>A), which results in skipping of exon 9. The homozygote phenotype of this mutation was very closely related to the phenotype of HMSNL/CMT 4D patients. HMSNL/CMT 4D is a hereditary autosomal recessive disease, resulting in demyelination of peripheral nerves. It is the most common form of demyelinating CMT disease in the Roma population. The disease results in severe disability in adulthood. It begins consistently in the first decade of life with a gait disorder, followed by upper limb weakness in the second decade and, in most subjects, by deafness setting in in the third decade of life. Sensory loss affecting all modalities is present; sensory loss and motor involvement predominates distally in the limbs. Skeletal deformity, particularly foot deformities, are frequent.

Implications in atherosclerosis

Patients with HMSNL were found to have a high total cholesterol: HDL-C ratio. Atherosclerosis is an important factor for the development of cardiovascular diseases, like myocardial infarction and angina pectoris. NDRG1 contributes to HDL-C (high-density lipoproteincholesterol) levels most likely by its phosphopantetheine-binding domain interacting with the high-density lipoproteins apolipoprotein A-I and AII.

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