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Review

N-myc downstream regulated 1 gene and its place in the cellular machinery

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The exact function of the protein product of N-myc downstream regulated 1 gene (*NDRG1*) is unclear. Depending on the tissue type the NDRG1 protein is localized in the cytoplasm, nucleus, mitochondrion or membranes. Moreover, the expression of *NDRG1* may be altered by several factors such as hypoxia, heavy metals, DNA damage, hormones, oncogene, and tumor-suppressor genes. A number of studies emphasize the role of NDRG1 in cancerogenesis. Presumably NDRG1 participates in angiogenesis, metastases, and mechanisms leading to anti-cancer drug resistance. This review summarizes current knowledge about the *NDRG1* gene and the position of NDRG1 protein in the cellular machinery. The role of NDRG1 in cancer pathogenesis and its possible usefulness as a prognostic factor for patients with cancer is also discussed.

Keywords: NDRG1, cancer, hypoxia, metastases, nickel

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INTRODUCTION

N-myc downstream regulated 1 gene (*NDRG1)*, also known as *Drg1, Cap43, Rit42, RTP* and *PROXY-1* was identified as a gene mutated in hereditary motor and sensory neuropathy-LOM (HMSNL; CMT4D) and mapped to human chromosome 8q24 (Kalaydjieva *et al.,* 1996). In this autosomal recessive neuropathy, demyelination is observed in both peripheral and central nervous system (Echaniz-Laguna *et al.,* 2007). The expression of *NDRG1* in peripheral nerves is markedly elevated in comparison to other tissue suggesting that this protein plays an important role in Schwann cells (Kalaydjieva *et al.,* 2000). Studies with knockout mice confirmed these assumptions. *NDRG1*$^{-/-}$ mice presented sporadic degeneration of the myelin sheath that began by 5 weeks of age. This indicates that NDRG1 is not necessary for the formation of the myelin sheath but is essential for its maintenance in the Schwann cells (Okuda *et al.,* 2004). Interestingly, the myelination in the brain of the *NDRG1*$^{-/-}$ mice was not altered suggesting that other members of the NDRG family compensate for the lack of NDRG in this part of central nervous system (Okuda *et al.*, 2008). *NDRG1*, *NDRG2, NDRG3* and *NDRG4* are members of the *NDRG* gene family belonging to the α/β hydrolase superfamily. Although the NDRG proteins have α/β hydrolase motifs, they lack the catalytic residues and have no hydrolytic activity (Shaw *et al.,* 2002). Proteins encoded by the *NDRG* genes show 52–65% amino-acid sequence homology to each other (Zhou *et al.,* 2001; Qu *et al.,* 2002) and are characterized by different patterns of tissue expression. *NDRG1* is ubiquitously expressed whereas *NDRG2* is mainly expressed in the brain and adult skeletal muscle, *NDRG3* transcript was found in the brain and testis, and *NDRG4* in brain and heart (Zhou *et al.,* 2001; Qu *et al.,* 2002). NDRG1 is a 43 kDa protein with an isoelectric point of pH 5.3, composed of 394 amino acids with three unique 10-amino-acid tandem repeats (GTRSRSHTSE) in the C-terminal region (Zhou *et al.,* 1998). It is a phosphoprotein with at least seven phosphorylation sites, and *in vitro* is directly phosphorylated by protein kinase A and calmodulin kinase II (Agarwala *et al.,* 2000; Sugiki *et al.,* 2004a). The NDRG1 protein has no evident signal sequence, a transmembrane domain, or an endoplasmic reticulum retention sequence (Kokame *et al.,* 1996). *NDRG1* mRNA is present in cells of most organ systems, including the digestive tract, immune, urinary and respiratory systems, however, its transcription level depends on tissue type. Immunohistochemical analysis showed a very strong NDRG1 signal in epithelial cells (Lachat *et al.,* 2002). The NDRG1 protein is mainly found in the cytoplasm, but nuclear, mitochondrial (Lachat *et al.*, 2002) and membrane (Bandyopadhyay *et al.*, 2004a; Nishio *et al.,* 2008) localization has also been reported. It was documented that NDRG1 translocates into the nucleus in a p53-dependent manner following DNA damage (Kurdistani *et al.,* 1998). Histochemical analysis of NDRG1 expression in gastric cancers showed that its nuclear localization correlated with worse prognosis for patients, suggesting that translocation to the nucleus leads to an increased cell invasion potential (Inagaki *et al.*, 2009). In some tissue only *NDRG1* transcript but not the protein was detected, implying the operation of a complex regulatory system controlling the amount of the protein both on the transcriptional and translational level (Lachat *et al.,* 2002). The presence of several CG-rich islands upstream of the *NDRG1* and results of studies with the use of an inhibitor of DNA methylation suggest that the expression of *NDRG1* is regulated by hypermethylation of the CpG islands (Guan *et al.,* 2000; Bandyopadhyay *et al.,* 2004a). Moreover, the *NDRG1* expression level has been reported to be dependent on the degree of histone acetylation (Guan *et al.,* 2000). Research performed in several laboratories has indicated that numerous compounds alter expression of *NDRG1*. It was reported that substances like homocysteine, cysteine, tunicamycin, 2-mercaptoethanol (Kokame *et al.,* 1996), phorbol myristate acetate, retinoic acid (Piquemal *et al.,* 1999; Chen *et al.*, 2009) and nickel (Zhou *et al.,* 1998; Salnikow *et al.,* 1999) induce *NDRG1* expression.

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Abbreviation: HER, hypoxia-responsive element

The exact function of NDRG1 is not clear because this protein interacts with many compounds and seems to be involved in cellular differentiation, response to hypoxia, and interaction with metals and hormones. It is also possible that NDRG1 takes part in carcinogenesis. The goal of the present review is to summarize current knowledge about of *NDRG1* gene and try to define the place of the NDRG1 protein in the cellular machinery.

FUNCTION OF NDRG1 IN THE CELL

It is difficult to determine the real biological function of NDRG1 protein, and numerous studies have demonstrated that the expression of the *NDRG1* gene differs under various situations. Northern blot analysis showed that *NDRG1* mRNA expression in normal cells is biphasic, i.e., it increases at the G and G2–M phases of the cell cycle and is lower in S phase (Kurdistani *et al.,* 1998). Other researches observed that NDRG1 is associated with the centrosome, specifically at the microtubule matrix, which suggests its role in the spindle checkpoint. Introduction of siRNA to knock down *NDRG1* expression in normal human epithelial cells increased the number of polyploid cells and disrupted spindle formation. Overexpression of *NDRG1* in p53-deficient human cancer cell lines exposed to a spindle checkpoint inhibitor (nocodazole) inhibited cell polyploidization and increased the number of cells in mitotic arrest, underlining NDRG1 importance in maintaining correct microtubule functioning, whose disturbance may lead to genomic instability (Kim *et al.,* 2004). Analyses of *NDRG1* relationships with known transcription factors provide important information necessary to understand the biological function of the *NDRG1* gene. Observations of mouse embryogenesis indicated that *Ndr1* (the mouse *NDRG1* homolog) is repressed by the N-myc:Max complex (Shimono *et al.,* 1999). Further experiments performed on human neuroblastoma cells have confirmed that expression of *NDRG1* is negatively regulated by N-myc. It was also demonstrated that another gene belonging to the MYC family, *c-Myc*, takes part in *NDRG1* repression (Li & Kretzner, 2003). The mechanism of the c-Myc suppressive action on *NDRG1* is not resolved definitely. Under *in vitro* condition the c-Myc dimerization and DNA-binding domain is able to weakly bind some parts of human *NDRG1* core promoter (Li & Kretzner, 2003), but other investigators propose that MYC proteins interact rather with other proteins forming the transcriptional complex (Shimono *et al.,* 1999; Zhang *et al*., 2008). Some observations point to the possible involvement of histone deacetylases in MYC-mediated repression of *NDRG1* transcription (Zhang *et al*., 2008). It is known that DNA damage leads to activation of *NDRG1* expression and induces translocation of NDRG1 protein to the nucleus, even though its amino-acid sequence does not contain any obvious nuclear localization signal (Kurdistani *et al.,* 1998). Stein and co-workers (2004) demonstrated that *NDRG1* is a direct target for 53 protein owing to a p53-binding site 406 bp upstream of the *NDRG1* transcriptional initiation site. An increase in *NDRG1* expression was observed not only following ectopic expression of p53, but also was evident upon treatment with the DNA-damaging drug doxorubicin which activates endogenous *TP53*. Interestingly, this relation was not observed in metastatic human cancer cell lines implying that in metastatic cells *NDRG1* is regulated differently than in non-metastatic cells. The same researchers noted that *NDRG1* expression is necessary for caspase 3 activation and is required for p53-dependent apoptosis (Stein *et al.,* 2004). A recent study performed on intestinal epithelial cells confirmed that *NDRG1* gene is a direct transcriptional target of p53, but also provided evidence that p53-dependent *NDRG1* expression suppressed cell proliferation and did not induce apoptosis (Zhang *et al.,* 2007). Among the proteins able to bind regulatory elements within the *NDRG1* promoter region is the chimeric transcription factor E2A-PBX1. This fusion protein is a result of the 1;19 chromosomal translocation occurring in some 5% of pediatric acute lymphoblastic leukemia cases. Studies performed on the murine bone marrow cell line Ba/F3 showed that overexpression of E2a-Pbx1 resulted in an increase of *NDRG1* gene expression followed by p53-independent cell apoptosis (Rutherford *et al.,* 2001). Therefore, it seems that the function of NDRG1 and its interaction with other proteins depend on the cell type.

As stressed above, the NDRG1 protein is predominantly found in the cytoplasm, but in some cell types nuclear localization was reported (Lachat *et al.,* 2002). However, no nuclear localization signal sequence has been indentified for this protein implying that translocation of NDRG1 to the nucleus depends on its interaction with other proteins. Work performed on murine mast cells has revealed that heat-shock cognate protein 70 (Hsc70) is able to bind the core domain of NDRG1 and translocate it to the nucleus (Sugiki *et al.,* 2004b). The binding of Hsc70 and NDRG1 was more efficient when the C-terminal tandem repeats of NDRG1 were deleted implying that the C-terminal part of NDRG1 might regulate the interaction of these two proteins. It is tempting to speculate that in response to a cellular stress (e.g., DNA damage) NDRG1 binds Hsc70 and is translocated to the nucleus where it interacts with DNA. The fact that Hsc70 together with auxilin takes part in uncoating of clathrin-coated vesicles (for review see: Zinsmaier & Bronk, 2001; Eisenberg & Greene, 2007) suggests that NDRG1 might be associated with the intracellular traffic.

NDRG1 IS A HYPOXIA-RESPONSIVE GENE

Oxygen deprivation results in the activation of cellular machinery that allows cell survival under decreased oxygen tension. Hypoxia inducible factor 1 (HIF-1) is a transcription factor that initiates the cascade of changes necessary to adapt the cell to hypoxic condition. This protein is a heterodimer composed of α subunit containing the active centre (HIF-1α, HIF-2α or HIF-3α, depending on tissue), and β subunit involved in DNA binding (for review see: Semenza *et al.,* 1999; Walmsley *et al.,* 2008). The *NDRG1* expression significantly increases in cells exposed to hypoxia. Examination of *NDRG1* gene organization has revealed two hypoxia-responsive elements (HRE) in its non-coding sequence at –1376 bp and –7503 bp (Le & Richardson, 2004). It is known that HIF-1 regulates the expression of a variety of hypoxia-responsive genes, like transferrin receptor gene (Lok *et al*., 1999), vascular endothelial growth factor receptor gene 1 (*Flt-1*) (Gerber *et al.,* 1997) and erythropoietin gene (*EPO*) by direct binding to their HER sequence (Madan & Curtin, 1993), therefore it was suspected that this manner of regulation might also be operational for *NDRG1*. Indeed, hypoxia-induced expression of *NDRG1* does not occur in HIF-1$\alpha^{-/-}$ fibroblasts, whereas it was observed in wild-type HIF-1$^{+/+}$ fibroblasts (Salnikow *et al*., 2002). However, during prolonged hypoxia,

two days at least, some NDRG1 protein appears in HIF-1-deficient cells as well (Cangul *et al.,* 2004). These results indicate that hypoxia induces *NDRG1* expression in a HIF-1-dependent and -independent manner. Moreover, hypoxic conditions increase the level of phosphorylated NDRG1 protein indicating that NDRG1 is subject to posttranscriptional regulation (Sibold *et al.*, 2007). Under normoxic conditions HIF-1α and HIF-2α are rapidly degraded in a von Hippel-Lindau (VHL) protein-dependent manner (Maxwell *et al.,* 1999; Ohh *et. al.*, 2000; Cockman *et al.*, 2000). The VHL protein acts as a recognition molecule of a ubiquitin ligase complex that targets associated HIF-α subunits to ubiquitin-dependent degradation in the proteasome. Work on human renal cancer cells showed that VHL-negative cells had increased expression of several hypoxia-inducible genes, including *NDRG1* (Masuda *et al.,* 2003). Introduction of the *VHL* gene to VHL-negative cells downregulated the *NDRG1* expression. Examination of the *NDRG1* promoter has demonstrated that an Sp1 site in the region from –286 to –62 base pairs was in part responsible for the VHL-induced suppression of *NDRG1*, however, a detailed mechanism of such regulation has not been resolved yet. Analysis of the murine *Ndr1* promoter region indicates that the Sp1 binding motif overlaps the early growth response 1 (Erg-1) transcription factor-binding site at the –80 to –45 bp region. The Egr-1 transcription factor belongs to a family of early response genes induced by hypoxia and other stress signals. Recent studies have revealed that Egr-1 protein directly binds to the Egr-1/Sp1 motif and mediates the hypoxia-induced expression of *NDRG-1* (Zhang *et al.*, 2007).

NDRG1 AND HEAVY METALS

Nickel, a well-known carcinogen and hypoxia-mimicking metal, induces expression of various genes necessary for the cell survival under unfavourable conditions. Initially it was reported that exposure of cells to non-toxic doses of Ni^{2+} compounds resulted in an increase of *NDRG1* expression (Zhou *et al.,* 1998). That study documented that with regard to metals the induction of *NDRG1* gene was nickel-specific. Although Zhou and co-workers did not find any Ni^{2+} binding motif within NDRG1, later studies demonstrated that a motif of 14 amino acids (TRSRSHTSEGTRSR) repeated three times in the C-terminal part of this protein contains a metal binding site (Zoroddu *et al.*, 2001). It should be stressed that those studies examined only a small fragment of the protein; therefore a physical interaction of the entire NDRG1 protein with Ni^{2+} remains uncertain. Li and Kretzner (2003) have demonstrated that the induction of *NDRG1* by nickel is associated with suppression of *N-myc* in neuroblastoma cells. N-myc is a known suppressor of *NDRG1*, so nickel might indirectly up-regulate *NDRG1* by inhibition of the negative regulator. Moreover, it is possible that nickel up-regulates the *NDRG1* gene by stimulation of some *NDRG1* inducers. It was observed that cell exposure to nickel resulted in an increase of HIF-1α protein content (Salnikow *et al.,* 1999; Andrew *et al.*, 2001; Barchowsky *et al.*, 2002). On the other hand, heavy metals including nickel alter the intracellular calcium homeostasis by targeting Ca^{2+} channels (Busselberg, 1995; Zamponi *et al.*, 1996; Bernal *et al.*, 1997; Tarabova *et al.*, 2006). It was observed that exposure of cells to nickel leads to an increased intracellular Ca^{2+} level and overexpression of *NDRG1* (Salnikow *et al.,* 1999). A further study showed that Ca^{2+}-induced *NDRG1* expression occurred in both HIF-1α positive and negative cells (Salnikow *et al.*, 2002). The mechanism of Ca^{2+}-induced *NDRG1* expression is not fully resolved. Experiments conduced by Salnikow and co-workers (2002) demonstrated that a Ca^{2+} ionophore strongly increased c-Jun protein level and activated AP-1-dependent transcription of the *NDRG1* gene, although the elevated intracellular Ca^{2+} neither induced the HIF-1α protein nor HIF-1-dependent transcription. AP-1-dependent activation of *NDRG1* gene was also inferred from a study on HeLa cells treated with the cell cycle suppressor mimosine (Dong *et al.,* 2005). On the other hand, in mouse epidermal C141 cells nickel compounds induced *NDRG1* expression in a HIF-1-dependent manner (Li *et al.*, 2004). That study demonstrated that nickel induced HIF-1 expression by the phosphatidyl inositol 3 kinase (PI3-K)/protein kinase B (Akt) pathway. However, others observed that the NDRG1 protein is a very efficiently phosphorylated by serum and glucocorticoid-induced protein kinase 1 (SGK1) and extremely poorly by other AGC protein kinase family members (Akt1 and two ribosomal S6 kinases: S6K1 and RSK1) (Murray *et al.*, 2004; 2005). These findings underline that *NDRG1* is regulated by a mechanism dependent on PI3K, but Akt kinase has a very small share in this pathway. On the other hand, SGK1 is activated by PDK-dependent phosphorylation which requires phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) and PI3K activity (Kobayashi & Cohen, 1999). Taken together, it might be assumed that nickel is a stress factor leading to an increased PIP3 level, which in turn results in activation of both Akt and SGK1 kinases. Akt induces HIF-dependent transcription of *NDRG1* and SGK1 phosphorylates the NDRG1 protein, possibly altering its function.

Some reports link the expression of *NDRG1* to intracellular iron level (Lee & Richardson, 2004; Dong *et al.*, 2005; Kovacevic *et al.*, 2008). Iron is required for many cellular processes and its deprivation results in G1/S arrest and apoptosis (for review see: Le & Richardson, 2002). It has been observed that exposure of cells to iron chelators results in an increase of *NDRG1* mRNA level. The iron chelators-dependent up-regulation of *NDRG1* expression appeared to be p53-independent and was observed in both HIF-1 positive and negative cells (Lee & Richardson, 2004). The mechanism of *NDRG1* suppression by iron remains unresolved. *NDRG1* does not contain consensus iron responsive elements (IREs), therefore iron does not bind directly to *NDRG1* regulatory elements.

NDRG1 AND CANCERS

The cellular function of NDRG1 remains elusive, it is therefore difficult to understand the role of the *NDRG1* gene in carcinogenesis. Researches try to explain the variability of *NDRG1* expression level in a wide variety of cancers by studying relationships between *NDRG1* and known oncogenes or tumor suppressor genes. It seems that *NDRG1* is up-regulated by a product of the *PTEN* (phosphatase and tensin homologue deleted on chromosome 10) tumor suppressor gene (Unoki & Nakamura, 2001; Bandyopadhyay *et al.,* 2004b). *PTEN* is one of the most common targets of mutation in human cancer, with a mutation frequency similar to that of *TP53*. PTEN inhibits PI3K-dependent activation of Akt, and deletion or inactivation of *PTEN* results in constitutive Akt activation (for review see: Cantley & Neel, 1999). PTEN, acting in opposition to PI3K, is connected with cell

death and cell cycle arrest (for review see: Dahia, 2000). Studies on prostate and breast cancers showed that expression of *NDRG1* significantly correlates with *PTEN* expression and that up-regulation of *NDRG1* occurs through an Akt-dependent pathway. It has been suggested that combined determination of both *PTEN* and *NDRG1* expression levels has a better predictive value for prostate and breast cancer patient outcome than either marker alone (Bandyopadhyay *et al.,* 2004b). Such a relationship was not observed in endometrial carcinoma. Examination of tissue samples derived from estrogen-related endrometroid carcinoma showed that most of NDRG1-positive tissue exhibited a decrease in *PTEN* expression and most of PTEN-positive cancers displayed decreased expression of *NDRG1* (Chen *et al.,* 2008; Li *et al.,* 2008). The NDRG1 protein is undetectable in normal endometrium, but its level increases during the secretory phase of the menstrual cycle, which is consistent with its differentiation-related function (Malette *et al.,* 2003). In endometroid carcinoma *NDRG1* overexpression is observed but does not correlate with the differentiation degree (Chen *et al.,* 2008; Li *et al.,* 2008). Therefore, in endometrial cancer factor(s) other than PTEN must participate in the control of *NDRG1* expression. The fact that the properties of endometrium are highly dependent on hormone level may be the clue to understand the NDRG1 functions. In breast and prostate cancer *NDRG1* expression has been reported to be susceptible to the hormonal status of environment. Ulrix *et al.* (1999) demonstrated that androgen indirectly induces *NDRG1* expression in prostatic and some breast carcinoma cell lines. Also Segawa *et al.* (2002) found *NDRG1* among genes up-regulated by androgen in prostate cancer cells. Similarly, estrogen treatment leads to *NDRG1* induction through estrogen receptor-α (ERα) in prostatic cells (Pflueger *et al.,* 2009). An opposite effect of estrogen on *NDRG1* expression was observed in ERα-positive breast cancer cell lines (Fotovati *et al.*, 2006). These different effects (down *vs* up-regulation) noted after action of the same factor (estrogen) through the same receptor (ERα) underline the tissue specificity of *NDRG1* function and/or regulation. Recent studies conducted on prostate cancer have revealed that *NDRG1* is fused to v-ets erythroblastosis virus E26 oncogene homolog (*ERG*) (Pflueger *et al.*, 2009). *ERG* encodes a transcription factor from the erythroblast transformation-specific (ETS) family and in prostate cancer it is often fused to the promoter region of the androgen-regulated transmembrane protease, serine 2 gene (*TMPRSS2*). Using the paired-end RNA-seq (RNA sequencing) method a new *NDRG1-ERG* fusion transcript was found. Hormone-induced overexpression of the *NDRG1-ERG* fusion may increase the level of functional ERG domains (Pflueger *et al.*, 2009). However, it is also possible that *ERG* fusion might disrupt the *NDRG1* gene leading to impaired function of NDRG1 protein.

NDRG1 is a hypoxia-associated gene and its expression should increase in tumor cells, which mostly function in an environment poor in oxygen and nutriments. Indeed, some studies have documented increased expression of hypoxia-induced genes in cancer cells (Cangul *et al.*, 2002a). Wang and associates demonstrated that NDRG1 protein level gradually increased during colorectal carcinogenesis and there was a significant difference in expression of *NDRG1* between non-metastatic and metastatic tumors (Wang *et al.*, 2004). On the other hand, experiments on tumor cell lines showed that *NDRG1* is down-regulated in cell lines descended from metastasis of colon cancer, and its overexpression leads to the induction of cell differentiation (Guan *et al.,* 2000). In another studies Shah *et al.* (2005) have examined colorectal liver metastases and found that the amount of NDRG1 was not significantly different between primary and metastatic lesion. On the other hand, *NDRG1* was found among genes induced early during *in vitro* maturation of mast cells, indicating its participation in cell differentiation (Taketomi *et al.*, 2003). Pancreatic cancer cell lines expressing relatively high level of *NDRG1* showed significantly lower invasive potential than cells with low *NDRG1* expression (Maruyama *et al.*, 2006). In those studies overexpression of *NDRG1* in pancreatic cancer cells did not disturb the growth rates in culture, but significantly reduced the rate of tumor growth in mice. Although similar experiments on prostate cancer cells showed no influence of *NDRG1* expression level on tumor growth, an association with lower incidence of lung metastases in animal models was observed (Bandyopadhyay *et al.,* 2003). Overexpression of *NDRG1* has been observed in human hepatocellular carcinoma and it correlated with tumor aggressiveness and poor patient survival (Chua *et al.*, 2007). The association of high NDRG1 level with the aggressiveness of hepatocellular cancer suggested a tumor-promoting role of NDRG1. Work performed by Yan and co-workers showed that silencing of *NDRG1* gene in hepatocellular carcinoma cell lines inhibited cell proliferation and invasion and induced apoptosis underlining that this protein has pro-survival and anti-apoptotic functions (Yan *et al.*, 2008). However, examination of a vide variety of tumor tissue has revealed that the expression level of *NDRG1* in cancer cells is usually similar or diminished in comparison with normal cells (Bandyopadhyay *et al.*, 2003; 2004a; Ando *et al.,* 2006; Strzelczyk *et al.*, 2009; Sun *et al.,* 2009). It was observed that low NDRG1 protein level was associated with worse prognosis for patients with glioma (Sun *et al.,* 2009), colorectal cancer (Pawelczyk *et al.*, 2007; Strzelczyk *et al.*, 2009), esophageal squamous cell carcinoma (Ando *et al.,* 2006), pancreatic ductal adenocarcinoma (Maruyama *et al.,* 2006), prostate cancer (Segawa *et al.,* 2002; Bandyopadhyay *et al.,* 2003), and breast cancer (Bandyopadhyay *et al.,* 2004a). The abovementioned reports indicate that, depending on cancer type, and sometimes on the analyzed group, expression of *NDRG1* correlates positively or negatively with the patients' survival and disease progression. The apparently contradictory findings regarding the *NDRG1* gene expression and its relation to cancer progression might indicate that *NDRG1* functions in a cell-specific manner and suggest that cancer-related factors alter the NDRG1 function in the cell. Moreover, there are reports indicating that the relation between NDRG1 protein expression and clinical outcome in cancers depends on the race/ethnicity of the patients. Studies conducted by Koshiji and colleagues documented that increased expression of *NDRG1* in colorectal cancer significantly correlated with aggressive phenotypes in the Japanese, whereas an opposite trend was observed in white patients (Koshiji *et al.*, 2007). Ethnic-dependent patterns of NDRG1 expression were also observed in prostate cancer patients in a large American study (Caruso *et al.,* 2004).

The observed relation between the level of NDRG1 protein and the potential of tumor cells to metastasize suggested that NDRG1 might interact with cell–cell adhesion molecules. Using immunoprecipitation followed by liquid chromatography-tandem mass spectrometry, Tu and co-workers found that NDRG1 protein binds directly to β-catenin and E-cadherin (Tu *et al.,* 2007). E-cadherin is a transmembrane glycoprotein that mediates

specific cell–cell adhesion in a Ca^{2+}-dependent manner, and a decrease in its expression is associated with cancer progression and development of metastases (for review see: van Roy & Berx, 2008). Another research team demonstrated that E-cadherin was up-regulated in cells overexpressing *NDRG1* (Guan *et al.*, 2000) and the levels of expression of those two genes were positively correlated in prostate cancer (Kachhap *et al.*, 2007). Moreover, Song *et al.* (2010) noted a significant correlation between the cellular localization of NDRG1 and E-cadherin. They observed that a decreased content of NDRG1 and E-cadherin in membranes of prostate cancer cells has a negative impact on patient survival and correlated with more advanced disease stages (Gleason score). Statistical analysis showed that the decline in membrane content of E-cadherin or NDRG1 was a poor prognostic factor but in multivariate analysis only the decrease of membrane-associated NDRG1 expression was a poor independent prognostic factor for patients with prostate cancer (Song *et al.*, 2010). Translocation of NDRG1 from the membrane to the cytoplasm might be important for the progression of prostate cancer, which appears to be under E-cadherin regulation. Further studies should be conducted to explain whether NDRG1 suppresses the cell invasion by interaction with E-cadherin, or whether these two molecules act independently.

The growth of a tumor depends on nutrient and oxygen availability determined by blood supply through new vessels developed in the tumor mass by means of angiogenesis. In some cancers high *NDRG1* expression was reported to correlate with angiogenic activity. In cervical adenocarcinoma increased expression of *NDRG1* associated with *VEGF* expression and angiogenesis, and correlated with poor overall survival (Nishio *et al.*, 2008). On the other hand, in pancreatic ductal adenocarcinoma high *NDRG1* expression closely associated with low angiogenesis and longer patients' survival (Maruyama *et al.,* 2006). Additionally, Maruyama and coworkers showed that production of angiogenic factors such as matrix metalloproteinase-9 (MMP-9), interleukin-8 (IL-8) and VEGF is markedly reduced in cell lines overexpressing *NDRG1*. However, solving the question whether NDRG1 plays any causative role in angiogenesis must await the results of future studies.

The sensitivity to anti-cancer drugs is one of the major points in clinical studies on tumor progression. Little is known about NDRG1 participation in changing the tumor sensitivity to treatment, but there is some evidence suggesting that it may be associated with this process. Studies on colon cancer cells showed that alteration of *NDRG1* expression changed their sensitivity to irinotecan (Motwani *et al.,* 2002). Irinotecan is a semi-synthetic analogue of camptothecin which inhibits topoisomerase I and is used in colorectal cancer therapy (for review see: Omyla-Staszewska & Deptala, 2003).

CONCLUSIONS

Data gathered to date indicate that the *NDRG1* gene is ubiquitously expressed in human tissue, including cancers. Its expression is subjected to complex regulatory pathways and is influenced by pleiotropic factors including hypoxia, oncogenes, tumor-suppressor genes, metal ions, and hormones. Therefore, it appears that *NDRG1* expression depends on the factor(s) predominating in a particular condition. Many studies indicate that the level of *NDRG1* mRNA and/or protein correlates with cancer progression. However, confirmation of the possible NDRG1 roles in cancer progression must await the results of future studies. Further observations on large groups of patients must be conducted to validate the NDRG1 cut point in specific tumor cases. For example, newly conducted analysis on primary colorectal cancers suggested that determination of *NDRG1* expression may be a useful tumor marker in selecting patients with low advanced disease who may benefit from adjuvant therapy (Strzelczyk *et al.*, 2009). Hopefully, in the near future we will gather data necessary to unravel the cellular function of NDRG1, and this in turn will lead to effective cancer therapy.

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