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Research Article

The Indolic Diet-Derivative, 3,3′-Diindolylmethane, Induced Apoptosis in Human Colon Cancer Cells through Upregulation of NDRG1

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N-myc downstream regulated gene-1 participates in carcinogenesis, angiogenesis, metastases, and anticancer drug resistance. In the present study, we analyzed the expression pattern of N-myc downstream regulated gene-1 following treatment of human colonic cancer cell lines; HCT-116 (well differentiated with wild-type p53 gene) and Colo-320 (poorly differentiated with mutant p53 gene), with 3,3′-diindolylmethane, a well-established proapoptotic agent product derived from indole-3-carbinol. Treatment of Colo-320 and HCT-116 with 3,3′-diindolylmethane disclosed inhibition of cell viability in a dose-dependent manner, mediated through apoptosis induction. The increased expression of N-myc downstream regulated gene-1 was detected only in poorly differentiated colon cancer cells, Colo-320 cell line. Our results suggest that N-myc downstream regulated gene-1 expression is enhanced by 3,3′-diindolylmethane in poorly differentiated cells and followed by induction of apoptosis. 3,3′-diindolylmethane induced apoptosis may represent a new regulator of N-myc downstream regulated gene-1 in poorly differentiated colonic cancer cells.

1. Introduction

N-myc downstream regulated 1 gene (NDRG1) is a 43-kDa protein located mainly in the cytoplasm but occasionally translocates into the nucleus in a p53-dependent manner [1]. This protein interacts with many cell elements and consequently participates in various processes in the cells, such as differentiation, response to stress conditions, involvement in carcinogenesis, angiogenesis, and metastasis. NDRG1 is induced, among others, by stress conditions, inhibition of DNA methylation, and histone deacetylation [2–7]. Furthermore, NDRG1 is necessary for caspase-3 activation and is required for p53-dependent apoptosis in a cell type-specific manner [8]. Nevertheless, the precise function of NDRG1 and its regulation in cancer cells is far from being clear.

In colon cancer, NDRG1 protein levels appear to be decreased with the progression from normal colonic epithelium to carcinoma [9, 10]. Moreover, NDRG1 expression is associated with a more aggressive tumor phenotype and poor outcome in colorectal cancer [11].

NDRG1 mediates apoptosis in several tissues [8, 12, 13]. However, the regulation of NDRG1 during apoptosis state in cancer cells in general, and in colon cancer in particular, was not explored previously. The purpose of the current study was to investigate the NDRG1 expression pattern in human colon cancer cell lines which vary in their differentiation level and in their p53 status. For apoptosis induction, we used 3,3′-diindolylmethane (DIM), a well-established proapoptotic agent product derived from indole-3-carbinol (I3C). In a low environmental pH, I3C is converted into polymeric products, among which 3,3′-diindolylmethane (DIM) is the main one. Studies showed that DIM induced cell-cycle arrest in human prostate cancer through, the upregulation of cycline-dependent kinases [14] and apoptosis via mitochondrial pathway [15–21].

2. Materials and Methods

2.1. Cell Culture and Treatment. Two human colon cancer cell lines HCT-116 (well differentiated, p53 wild type) and Colo-320 (poorly differentiated, p53 mutant) were grown in a medium 10% fetal calf serum and 100 U/mL penicillin-streptomycin in the presence of DIM (10–100 μM). Cell viability (XTT) [22], cytotoxicity, and leakage of lactate dehydrogenase [23, 24] were tested 24, 48, and 72 hours after treatment.

DAPI staining for visualization of chromatin condensation [25] were conducted after treatment with 60 μM DIM for 72 hr [26].

2.2. RNA Isolation and Real-Time PCR. RNA from colon cancer cells was isolated using Tri-reagent (Tal-Ron). Total RNA (3 μg) was reverse-transcribed to cDNA. 1197 bp product of NDRG1 was amplified by real-time PCR (ABI Prism 7900) using probe and the following primers: 5′-AGGGACATGTCTCGGGAGATGCAGGAT-3′ and 5′-GGCCGCCTAGCAGGAGACCTCCATGG-3′. As a control gene, we amplified β-actin (838 bp) with 5′-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3′ and 5′-CGTCATACTCCT GCTTGCTGATCCACATCTGC-3′.

Alkaline phosphatase activity assay [27] was conducted 4 and 8 h after treatment with 60 μM DIM.

2.3. Statistical Analysis. Data analyses were carried out using SPSS version 17. Experiments were repeated three times, each performed in triplicate, and the data are presented as means ± SD (Standard Deviation). Statistical analysis of the differences between controls and treated groups were performed using Student's t-test. $P < 0.05$ was considered significant.

3. Results

3.1. DIM Reduced Cell Viability by Apoptosis Induction. We observed that the two colon cancer cells treated with DIM (10–80 μM) for 72 hr had a significant inhibitory effect ($P < 0.001$) in a dose-dependent manner (Figure 1). The IC_{50} values were 52 μM for HCT-116 and 56 μM for Colo-320. Furthermore, no toxic effects were manifested for these concentrations, as it was determined with LDH enzyme activity (Data not shown).

Following the reduced viability, apoptosis was tested in cells treated with DIM (60 μM) for 72 hr (Figure 2(a)). Both cell lines showed DNA fragmentation, a main feature of apoptosis occurrence. In addition, cell treatment with 60 μM DIM for 48 h followed by DAPI staining (Figure 2(b)) showed apoptotic cells that exhibited typical apoptotic morphology, condensation, and fragmentation of the nucleus, in comparison to untreated cells.

3.2. Alteration in NDRG1 Expression in Colo 320. Basal expression of NDRG1 prior treatment showed low levels of mRNA and no expression of protein in Colo 320 cell lines; alternatively, high levels of both mRNA and protein were detected in HCT-116 cell line (Data not shown).

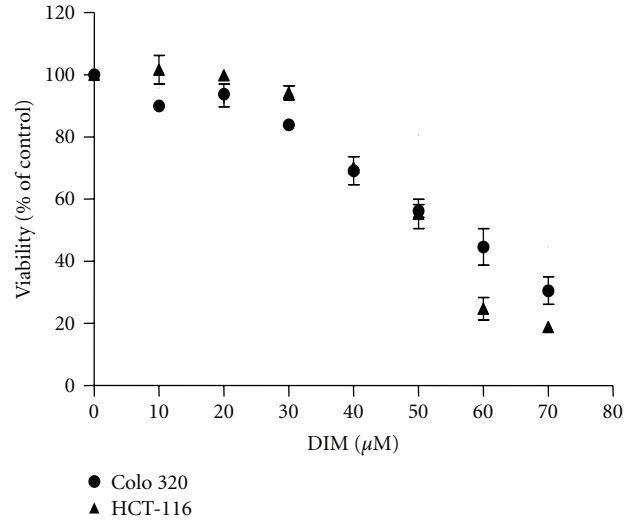

Figure 1: Effect of DIM on cell viability. Exponentially growing cells were incubated in the presence of 10 to 100 μM of DIM for 72 hours, and the viable cells were detected by XTT assay. The results are presented as percentage of control and expressed as means ± SD of three independent experiments.

Exposure of the Colo 320 to DIM for 24–72 hr resulted in a significant increase (9-fold for 24 h, 10-fold for 48 h, and 15-fold for 72 h following treatment) of the NDRG1 mRNA levels (Figure 3). However, exposure of HCT-116 to DIM resulted in uninfluenced expression of the NDRG1 mRNA compared to control cells.

3.3. Effect of DIM and NDRG1 Expression on Cell Differentiation. Alkaline phosphatase activity, a molecular marker of colon epithelial cells differentiation was used to determine whether the increase in NDRG1 expression is related to differentiation alteration in the cells. The results revealed that treated cells had a nonsignificant increase of alkaline phosphatase activity in Colo 320 cells and had no effect in HCT-116 cells (Figure 4).

4. Discussion

In the present study, we examined NDRG1 expression in human colon cancer cells during apoptosis induced upon DIM application. First, we showed that indol derivative found in crucifers (DIM) induced apoptosis in both human colon cancer cells HCT-116 and Colo 320. We have previously shown that DIM has a suppressive effect on the growth of breast cancer and prostate cell lines [15, 16, 20]. The IC_{50} values for DIM on growth inhibition of colon cancer cells (50–60 μM) were similar to those found for prostate and breast cancer cells (20–40 μM and 17–30 μM, resp.) [15, 28]. DIM affected the viability of the cancer cell in a time- and dose-dependent manner irrespective of their p53 phenotype. The effect was mediated by apoptosis as shown morphologically and by DNA fragmentation. Thus, colonic

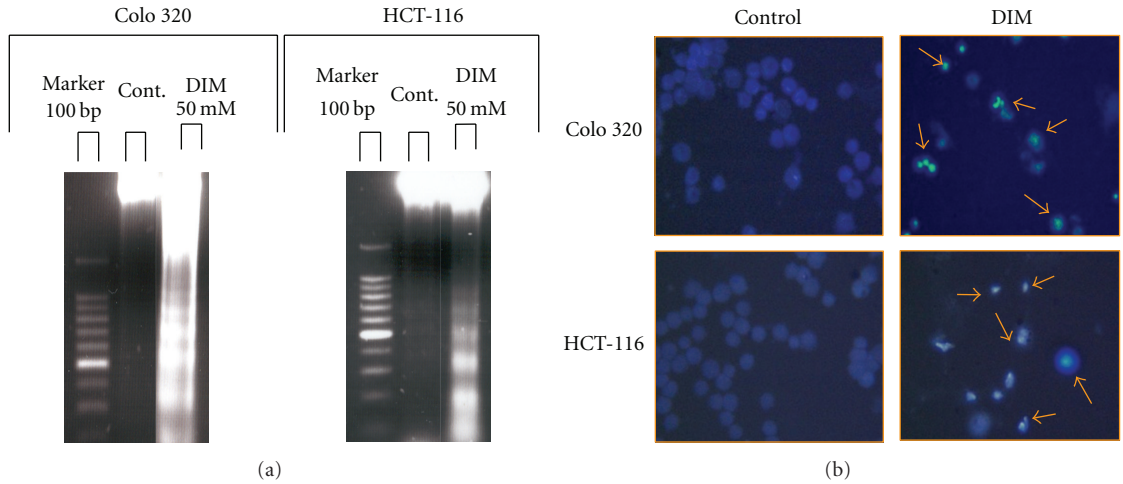

Figure 2: Apoptosis formation by DIM in Colo 320 and HGT116 cell lines. Cells were treated with DIM 60 μM for 72 hours. DNA was extracted and separated on a 1.5% Agarose gel. The data shown were repeated three times (a). Morphologic changes, denoting apoptotic cells were shown by cell morphological changes. Cells were incubated in the presence of 60 μM DIM for 48 hours and stained with DAPI. Morphologic changes are shown by arrows (b). The data shown were repeated three times.

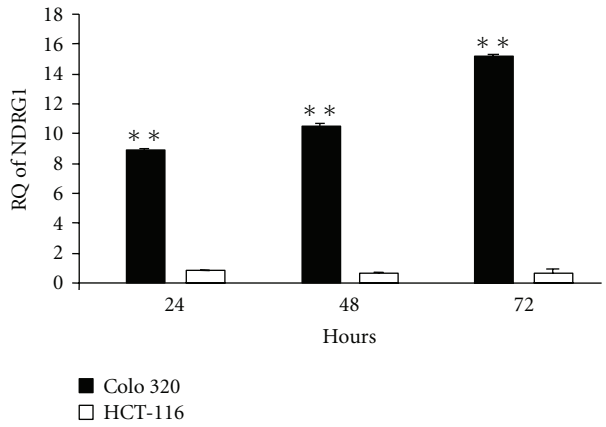

Figure 3: Expression of *NDRG1*. Cells treated with 60 μM DIM for 24, 48 and 72 h. The levels of mRNA were examined by real-time PCR and are represented as RQ (relative quantification) = 2^{-ddCt}. $^{*}P$ value <0.01, $^{**}P$ value <0.001.

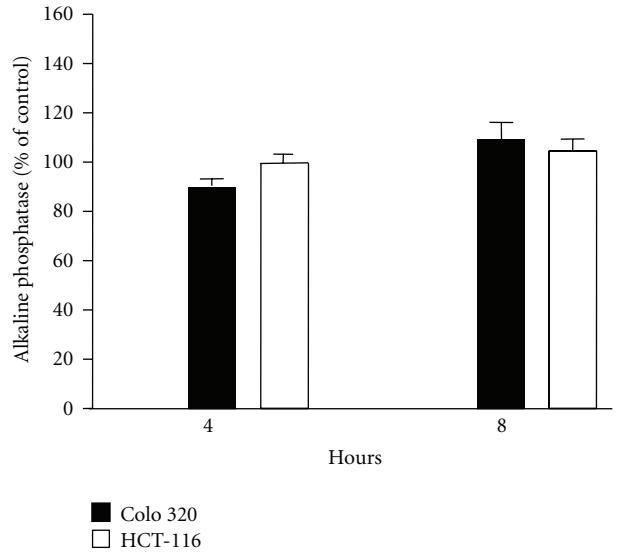

Figure 4: DIM treatment effect on alkaline phosphatase activity. Alkaline phosphatase activity (differentiation factor of colon cells) was detected after 4 and 8 hours of treatment. The results are presented as percentage of control and expressed as means ± SD of three independent experiments. $^{**}P$ value <0.001.

cell lines can be added to an increasing list of other organs' cell lines, namely, prostate and breast [16, 21, 29] that are affected by environmental factors which induce apoptosis.

The basal expression of NDRG1 reported here, high levels in well-differentiated cells (HCT-116) and low levels in poorly differentiated cells (Colo-320), was reported in colon [30], gastric cancer specimens [31], and in esophageal squamous cell carcinoma [32].

Quantization of NDRG1 expression showed that NDRG1 mRNA is increased 9–15-fold ($P < 0.001$) during treatment of the Colo-320 cells with DIM for 24–72 hr compared to control cells, indicating NDRG1 upregulation during apoptosis induced by DIM.

Up to now, it was known that NDRG1 expression is induced by DNA damage in a p53-dependent fashion, and it is necessary but not sufficient for p53-mediated caspase activation and apoptosis [8]. The present study shows for the first time that DIM increased NDRG1 expression in a p53 independent pathway followed by induction of apoptosis.

In conclusion, in this study, we provide evidence that the indole diet-derived DIM induce apoptosis in two human colon cancer cell lines, well- and poorly differentiated. However, induction of apoptosis by DIM is followed by increased NDRG1 expression only in a poorly differentiated and p53 mutant cells. On the other hand, DIM induced apoptosis in well-differentiated cells in a NDRG1-independent pathway. Our results may contribute to a better understanding of the

molecular mechanisms by which DIM exerts its effects in colon cancer cells and tumors. It is suggested that NDRG1 is not only a differentiation factor but might impact the apoptotic process. In all the NDRG1 transfection studies, apoptotic effects should be taken in account. In fact, NDRG1 is on the list of metastatic-related/cancer suicide genes drug development in the battle against cancer [33, 34]. Further studies are needed in order to examine NDRG1 role in colon cancer genesis. Results from the present research may contribute to the development of new and efficient strategies for colon cancer therapy.

Conflict of Interests

The authors declare no potential conflict of interests with respect to the authorship and/or publication of this paper.

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