



Targeted Overexpression of NDRG2 using Survivin Promoter Reduces Viability and Invasiveness of A549 Cell Line

Maryam Fanian ^{1,2}, Gholamreza Rafiei ³, Marzieh Alizadeh Zarei ¹ and Mohammad Ali Takhshid ^{1,3*}

1. Division of Medical Biotechnology, Department of Laboratory Sciences, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

2. Department of Infectious Diseases and Public Health, City University of Hong Kong, Kowloon, Hong Kong SAR 999077, China

3. Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

Abstract

Background: Anti-tumor effects of N-myc Downstream Regulated Gene2 (NDRG2) have been demonstrated in many tumors. In the present study, NDRG2 was specifically overexpressed in lung cancer cell line using Survivin Promoter (Sur-P). Then, the effects of NDRG2 overexpression on viability, apoptosis, migration, and invasion of A549 cells were evaluated.

Methods: Recombinant pAdenoVator-Sur-P-NDRG2-IRES-GFP plasmid harboring NDRG2 gene under transcriptional control of Sur-P and mock plasmid were constructed. A549 lung tumor cells and LX-2 cells (non-tumor cell line) were transfected with pAdenoVator-Sur-P-NDRG2-IRES-GFP, pAdenoVator-CMV-NDRG2-IRES-GFP, or mock plasmids. Tumor specificity of Sur-P was evaluated using fluorescent microscopy for GFP expression. The effects of NDRG2 overexpression on cell viability, apoptosis, and migration of A549 cells were measured using MTT, annexinV/7-AAD flow cytometry, and transwell migration assay, respectively. NDRG2 and matrix metalloproteinase-2 (MMP-2) expression were measured using real time-PCR.

Results: pAdenoVator-Sur-P-NDRG2-IRES-GFP transfection resulted in a huge GFP expression in A549 cells, but not in LX-2 cells. The results of real time-PCR analysis also showed that pAdenoVator-Sur-P-NDRG2-IRES-GFP transfection led to an abundant NDRG2 expression in A549 cells. NDRG2 overexpression decreased A549 cell viability through increasing cell apoptosis. In addition, migration, invasion, and MMP-2 expression decreased following NDRG2 overexpression in A549 cells.

Conclusion: The findings indicate that the targeted overexpression of NDRG2 using Sur-P can reduce the viability and invasiveness of A549 cells, suggesting possible benefits of this approach in lung cancer therapy.

Keywords: A549 cells, Apoptosis, Lung cancer, Matrix metalloproteinase 2, Metastasis, Survivin

To cite this article: Fanian M, Rafiei Gh, Alizadeh Zarei M, Takhshid MA. Targeted Overexpression of NDRG2 using Survivin Promoter Reduces Viability and Invasiveness of A549 Cell Line. Avicenna J Med Biotech 2023;15(2):84-90.

* Corresponding author:
Mohammad Ali Takhshid, Ph.D.,
Division of Medical
Biotechnology, Department of
Laboratory Sciences, School of
Paramedical Sciences, Shiraz
University of Medical Sciences,
Shiraz, Iran,
Diagnostic Laboratory Sciences
and Technology Research Center,
School of Paramedical Sciences,
Shiraz University of Medical
Sciences, Shiraz, Iran
Tel: +98 917 3121699
E-mail:
takhshidma@sums.ac.ir
Received: 20 Jul 2022
Accepted: 24 Jan 2023

Introduction

According to GLOBOCAN 2020 statistics, lung cancer is the second in incidence (11.4%) and the first in mortality rate (18%) among 36 prevalent cancers worldwide ¹. Histologically, lung cancer is classified as Small-Cell Lung Carcinoma (SCLC) and Non-SCLC (NSCLC), which account for about 15% and 85% of lung cancer cases, respectively ². Surgical resection, radiotherapy, chemotherapy, targeted therapy, and combination therapy are available therapeutic strategies for lung cancer. Four classes of chemotherapeutic

drugs are used for the treatment of patients with NSCLC which include alkylating agents (cisplatin and carboplatin) microtubule-targeting drugs (paclitaxel and docetaxel), antimetabolites (pemetrexed and gemcitabine), and topoisomerase inhibitors (etoposide) ³. Although chemotherapy still plays an important role in the treatment of patients with lung cancer, drug toxicity and resistance are the main challenges in this issue. Previous studies have revealed the role several mechanisms in developing drug resistance including reduced

drug uptake to tumor cells, increased drug efflux from the tumor cells, genetics and epigenetic modifications, apoptosis dysfunction, and acquisition of cancer stem cell-like properties ⁴. Therefore, finding an efficient and safe therapeutic method that specifically targets cancer cells has been the focus of many studies. In this context, gene therapy has emerged as a promising approach ⁵.

Tumor Specific Promoters (TSPs), transcriptional regulatory elements that ensure targeted and efficient expression of genes in tumor cells, are key elements in the success of gene therapeutic approaches ⁶. Survivin, an apoptosis inhibitory protein, is expressed in numerous types of cancer including SCLC and NSCLC ⁷. Numerous studies have revealed tumor specificity and strength of Survivin promoter (Sur-P) in the expression of various genes in several tumor cells ⁸. Moreover, its transcriptional activity is increased in hypoxia, a condition that usually found in tumor cells ⁹. Specific overexpression of SP1 protein, a transcription factor that directly binds to Sur-P, results in high transcriptional activity of Sur-P in lung tumor cells ¹⁰.

N-myc Downstream Regulated Gene 2 (*NDRG2*) is a multifunctional protein with crucial roles in the regulation of cell growth and differentiation. *NDRG2* expression is downregulated in many cancers including lung cancer. Furthermore, its expression has been found to be inversely associated with tumor stage, tumor size, and invasion of tumor cells to circulation and directly associated with patient's survival time ^{11,12}. Moreover, Faraji *et al* showed that *NDRG2* upregulation in A549 cells suppressed cell viability and invasion of tumor cells through decreasing expressions of Matrix Metalloproteinases-2 and -9 (*MMP-2* and *MMP-9*) ¹³.

The present study aimed to construct a recombinant plasmid harboring *NDRG2* cDNA under the transcriptional control of Sur-P to evaluate the tumor specificity and strength of this promoter in enhancing the *NDRG2* expression. The results revealed specific overexpression of *NDRG2* in A549 cell lines using this plasmid. Furthermore, *NDRG2* overexpression was associated with increased apoptosis and decreased invasiveness of the tumor cells.

Materials and Methods

Construction of plasmids

Three types of recombinant plasmids were used in this study including pAdenoVator-CMV-*NDRG2*-IRES-GFP (*NDRG2* under transcriptional control of CMV promoter), pAdenoVator-Sur-P-*NDRG2*-IRES-GFP (*NDRG2* under transcriptional control of Sur-P), and pAdenoVator-Sur-P-IRES-GFP (Mock plasmid). CMV-*NDRG2* was constructed in our previous studies ^{24,26}. To construct Sur-P-*NDRG2* plasmid, Sur-P ¹⁴ was amplified from genomic DNA (prepared from peripheral blood mononuclear cells) using forward (5'-CCG GAGTCGACGTTCTTTGAAAGCAG-3'; contained

SalI restriction site) and reverse (5'-TTAGCTCTAGA CAACGTCGG GGCACC-3'; contained XbaI restriction site) primers. Sur-P fragment was digested by XbaI and SalI restriction enzymes (Thermo Scientific, USA) and cloned into the pAdenoVator- IRES-GFP vector using T4 DNA ligase (Thermo Scientific, USA). The ligation mixture was then transformed into competent *Escherichia coli* (*E. coli*) DH5 α . The formation of Sur-P-*NDRG2* was validated using colony PCR and sequencing.

Cells and cell culture conditions

A549 cell line (ATCC ref. No. CLL-185) was purchased from Pasture Institute, Iran. LX-2 (human hepatic stellate cell line) was kindly provided by Dr. Younes Hoseini. A549 cells and LX-2 cells were cultured in RPMI 1640 (Gibco/Invitrogen, Carlsbad, CA) supplemented with 10% and 5% Fetal Bovine Serum (FBS) (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). Both cell lines were incubated at 37°C with 5% CO₂ atmosphere.

Overexpression of the *NDRG2* gene in A549 cells

To evaluate tumor specific activity of Sur-P, A549 and LX-2 cell lines were transfected with pAdenoVator-CMV-*NDRG2*-IRES-GFP, pAdenoVator-Sur-P-*NDRG2*-IRES-GFP, and mock plasmids using lipofectamine™ 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After 48 hr, the transfected cells were detached using 0.25% Trypsin-EDTA, washed, and resuspended in cold PBS buffer. Fluorescence microscopy and flow cytometry analyses were used to monitor the cellular expression of GFP and to measure the efficiency of transfection. Fluorescence microscopy was performed on an inverted microscope (Hund, Germany) with filter sets designed for GFP. Flow cytometric analysis was conducted using BD FACSCalibur flow cytometer (BD Biosciences, USA). For each sample, 10,000 events were collected. The expression of *NDRG2* was evaluated using real-time PCR as described in the following section.

Cell viability assay using the MTT method

The effect of *NDRG2* overexpression on cell viability was evaluated using MTT assay. Briefly, 6×10³ A549 cells were seeded on each well of a 96-well plate and were transfected with either pAdenoVator-Sur-P-*NDRG2*-IRES-GFP plasmid (*NDRG2* group) or mock plasmid (pAdenoVator-Sur-P-IRES-GFP) using Lipofectamine 2000 (Invitrogen, USA). Forty-eight hours after transfection, the cells were treated with 10 μ l of MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] solution followed by a four-hour incubation. Afterwards, the media were removed, formazan crystals were dissolved by Dimethyl Sulfoxide (DMSO), and the absorbance was measured at 545 nm.

Cell apoptosis assay

Annexin V/7AAD flow cytometry was used to evaluate the effect of *NDRG2* overexpression on cell apoptosis. In doing so, A549 cells were transfected with

NDRG2 or mock plasmids, as described previously. Forty-eight *hr* after transfection, the cells were harvested using trypsin digestion (0.25%) and centrifugation. The cells were then washed with ice-cold PBS, suspended in 1× annexin V binding buffer, and stained with PE annexin V and 7-AAD at room temperature in the dark for 15 *min*. The percentages of live and apoptotic cells were determined using the FACSCalibur instrument (BD Bioscience, USA) and flowjo software analysis.

Migration and invasion assay

Transwell migration assay was applied using 24-well transwell insert and 8 μ m pore filters (BD Bioscience, Bedford, MA) to evaluate the effects of NDRG2 overexpression on the migration of the transfected A549 cells. Briefly, 48 *hr* after transfection, A549 cells (5×10^4) were suspended with a serum-free medium, kept serum starved for 24 *hr*, transferred to the upper part of the transwell, and incubated at 37°C. The cells were then allowed to migrate to the lower part of the transwell, which was filled with RPMI supplemented with 10% FBS. After 24 *hr* incubation, the cells that migrated to the lower surface of the transwell were fixed with methanol for five minutes and were stained with 0.1% crystal violet. For invasion assays, the transwell was coated with 100 μ l (1 *mg/ml*) matrigel (BD Bioscience, Bedford, MA). Forty-eight *hr* after transfection, A549 cells (5×10^4) were plated onto the upper part of the matrigel-coated transwell chamber and were incubated for 48 *hr*. The invaded cells were then fixed with methanol, stained, and counted. The number of invading or migrating cells was determined by counting five fields (400×) of a light microscope on each membrane and was calculated as the mean number of cells per field^{15,16}.

Real time polymerase chain reaction

A549 and LX-2 cell lines were cultured at 1×10^5 and 1.5×10^5 counts in six-well plates, respectively. Both cell lines were transfected with pAdenoVator-Sur-P-NDRG2-IRES-GFP. Forty-eight *hr* after transfection, the cells were exposed to 1 *ml* TRIzol (Thermo Fisher Scientific, USA) per 1×10^6 cells and the RNAs were extracted according to the manufacturer's protocol. After that, cDNAs were synthesized from the extracted RNAs using Takara kit according to the manufacturer's instructions. Quantitative real-time PCR was performed using specific primers (Table 1), SYBR-

Green master mix, and the Rotor-Gene Q (Qiagen, Germany) in a total reaction volume of 20 μ l.

Statistical analysis

All statistical analyses were performed using the SPSS software, version 15.0. Statistical differences between the study groups were analyzed using Mann-Whitney or Kruskal-Wallis test. The data represented mean $\pm$ Standard Deviation (SD) of at least three independent experiments. The $p < 0.05$ was considered statistically significant.

Results

Tumor specificity of survivin promoter

Tumor specific activity of Sur-P was evaluated using fluorescence microscopy (Figure 1 A-D). As illustrated in figure 1A, transfection of pAdenoVator-CMV-NDRG2-IRES-GFP plasmid into normal LX-2 cells led to a high GFP expression (Figure 1A), while transfection with pAdenoVator-Sur-P-NDRG2-IRES-GFP did not lead to GFP expression (Figure 1B). In A549 cells, however, transfection with both pAdenoVator-CMV-NDRG2-IRES-GFP (Figure 1C) and pAd-

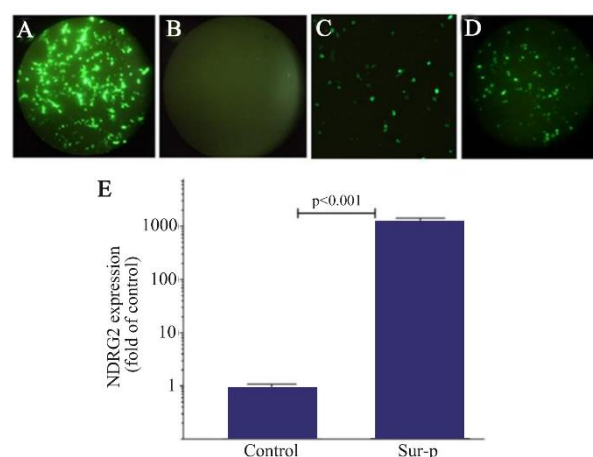


Figure 1. Tumor specificity of Green Fluorescent Protein (GFP) expression under Sur-P control. GFP was expressed in the LX-2 cells transfected with pAdenoVator-CMV-NDRG2-IRES-GFP (A), but not in those transfected with pAdenoVator-Sur-P-NDRG2-IRES-GFP (B). GFP was also expressed in the A549 cells transfected with both pAdenoVator-CMV-NDRG2-IRES-GFP (C) and pAdenoVator-Sur-P-NDRG2-IRES-GFP (D). Real-time PCR data (E) of NDRG2 expression in the A549 cells transfected with the pAdenoVator-Sur-P-NDRG2-IRES-GFP plasmid and control non-transfected cells. The data represent the mean $\pm$ SD of at least three independent experiments.

Table 1. The primer sequences for the real-time PCR analysis of NDRG2 and MMP-2 gene expressions

Primer	Sequence	Product length (bp)
NDRG2 (forward)	5'-CCTCACCTCTTCCATTCC-3'	188
NDRG2 (reverse)	5'-TATCACCTCCACGCTCAA-3'	
MMP2 (forward)	5'-TGGAGATACAATGAGGTGAAGAAG-3'	261
MMP2 (reverse)	5'-GAAGGCAGTGGAGAGGAAG-3'	
B-actin (forward)	5'-GCACAGAGCCTCGCCTTT-3'	230
B-actin (reverse)	5'-GCCTCGTCGCCCACATAG-3'	

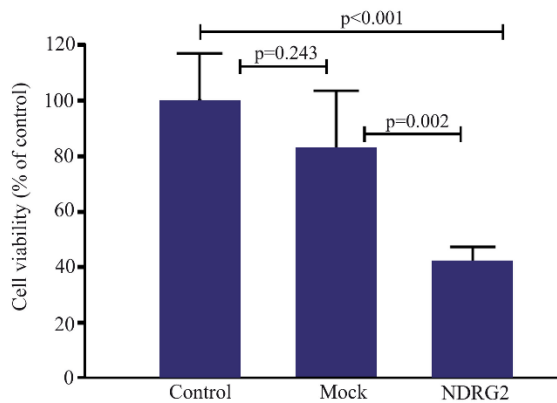


Figure 2. MTT assay results showing the effects of *NDRG2* overexpression on A549 cell viability. A549 cells were transfected with pAdenoVator-Sur-p-NDRG2-IRES-GFP or mock plasmids and cell viability was evaluated using the MTT assay. The data were analyzed using Kruskal-Wallis test. The data represent the mean±SD of at least three independent experiments.

enoVator-Sur-P-NDRG2-IRES-GFP (Figure 1D) led to *GFP* expression, suggesting tumor specificity of Sur-P. The results of real-time PCR also revealed an abundant *NDRG2* expression in the A549 cells (1200-fold) transfected with the pAdenoVator-Sur-P-NDRG2-IRES-GFP plasmid compared to the control non-transfected cells, suggesting the high strength of Sur-P in inducing *NDRG2* expression in A549 tumor cells.

Cell viability assay

Figure 2 shows the results of MTT assay. The data revealed that *NDRG2* overexpression decreases the viability of A549 cells compared to the control non-transfected cells ($p<0.001$) and the mock plasmid-transfected group ($p=0.002$). No significant difference was observed between the viability of control and mock groups ($p=0.243$).

Flow cytometric analysis of apoptosis

As shown in figure 3, *NDRG2* overexpression increased the percentage of total apoptotic cells compared to the mock-transfected ($p=0.047$) and control non-transfected cells ($p=0.039$). However, a decrease was detected in cell viability following *NDRG2* overexpression compared to the mock and control non-transfected cells.

Migration and invasion assay

Figure 4 illustrates the effects of *NDRG2* overexpression on the migration and invasion of the A549 cells. Based on the data presented in figure 4, *NDRG2* overexpression reduced the number of migrated and invaded cells compared to the mock and control cells, suggesting the anti-metastatic effects of *NDRG2* on A549 cells.

The effect of *NDRG2* overexpression on the level of MMP-2 mRNA

The effect of *NDRG2* overexpression on MMP-2 expression was evaluated using real-time PCR. The

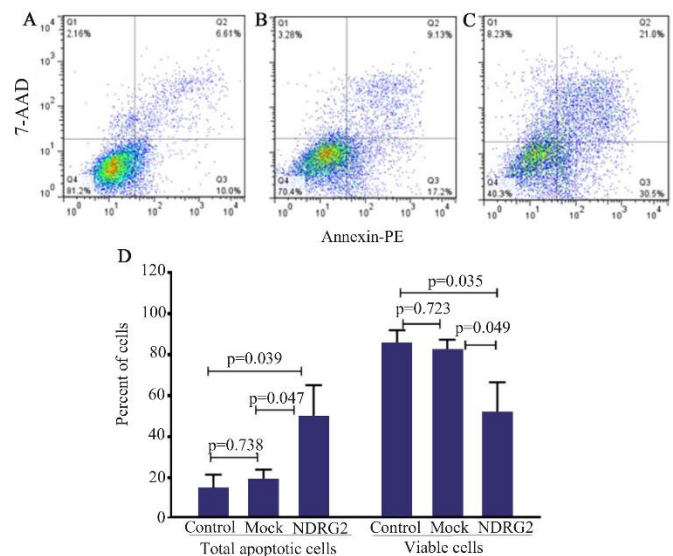


Figure 3. The effect of *NDRG2* overexpression on the percentage of live and total apoptotic A549 cells. The charts show the percentages of live cells (Q4), early apoptotic cells (Q3), late apoptotic cells (Q2), and necrotic cells (Q1) in the control (A), mock (B), and *NDRG2* (C) groups. The data (D) represent the mean±SD of three independent experiments. The data were analyzed using Kruskal-Wallis's test.

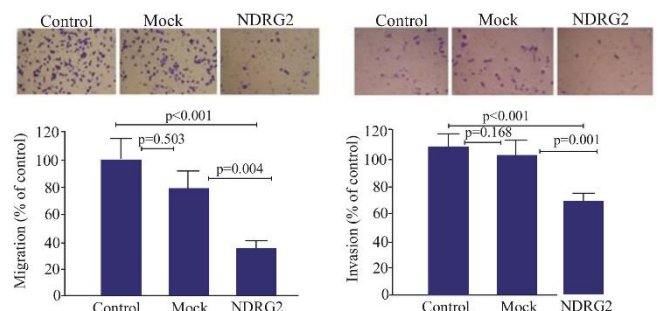


Figure 4. The effects of *NDRG2* overexpression on the migration and invasion of A549 cells using transwell assay. The percentage of migrated and invaded A549 cells in the mock plasmid transfected (mock), pAdenoVator-Sur-P-NDRG2-IRES-GFP-transfected (*NDRG2*), and control non-transfected cells. The data represent the mean±SD of three independent experiments. The data were analyzed using Kruskal-Wallis test.

results revealed a significant decrease in the MMP-2 mRNA level ($p=0.045$) compared to the control non-transfected cells (Figure 5).

Discussion

Several studies have revealed the beneficial effects of various gene therapy methods including inhibition of oncogenes and replacement of tumor-suppressor genes in the treatment of lung cancer ⁵. However, lack of tumor specificity is the most important limitation of these approaches. Two main strategies have suggested for increasing tumor specificity of gene therapy including targeted transfer to tumor cells *via* tumor specific

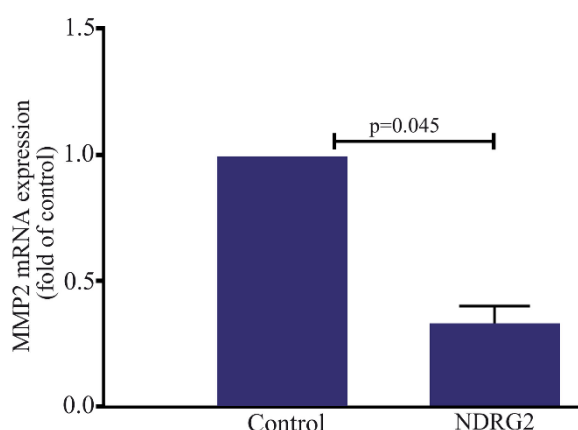


Figure 5. *NDRG2* overexpression reduced *MMP-2* expression in the A549 cell line. The data represent the mean $\pm$ SD of at least three independent experiments. The data were analyzed using Mann-Whitney test.

* $p < 0.05$ was considered statistically significant

receptors and targeted transcription of anti-tumor genes using TSPs. Despite the tumor specificity of various TSPs, low transcriptional efficiency usually hinders their utilization for gene therapy strategies. The present study assessed the specificity and efficiency of Sur-P in the overexpression of *NDRG2* in A549 cells. The findings revealed an abundant expression of *NDRG2* under the transcriptional control of Sur-P in the lung cancer cell line, but not in the normal cell line, compared to the CMV promoter that enhanced *NDRG2* overexpression in both normal and tumor cells. Furthermore, *NDRG2* overexpression decreased cell viability and invasiveness of A549 cells, suggesting specificity and efficiency of this strategy and its possible effectiveness in the treatment of NSCLC.

Survivin is anti-apoptotic protein that is specifically expressed in the most of human cancers including lung cancer. Numerous studies have revealed that efficient downregulation of survivin using transcriptional and post-translational approaches resulted in death and apoptosis of tumor cells and sensitized tumor cells to chemotherapeutic regimens¹⁷. In addition, several studies have described the beneficial use of Sur-P for the targeted expression of anti-tumor genes¹⁸. Bao *et al* used a 1092-bp fragment of the human Sur-P to control the expression of alkaline phosphatase in several tumor cell lines and reported its promoter activity in all evaluated tumor cells¹⁹. However, the large size of the fragment hampered its use for the construction of recombinant plasmids⁸. Therefore, Yang *et al* evaluated the efficiency of a number of surviving promoter fragments and indicated that a 269-bp fragment of Sur-P was sufficient for the specific and efficient expression of genes in human pancreatic and breast cancer cell lines¹⁴. In the current study, we constructed a plasmid harboring Sur-P and human *NDRG2* cDNA. Our data revealed that Sur-P can mediate *NDRG2* overexpres-

sion in A549 cells, but not in LX-2 cells, suggesting the tumor specificity and strength of this promoter in lung cancer cells. LX-2 has been used in some studies to determine tumor specificity of some promoters^{20,21}. Nonetheless, LX-2 is a hepatic stellate cell line and the lack of evaluation of Sur-P activity in the lung epithelial cells is among the limitations of this study. Further studies are needed to determine transcriptional activity of Sur-P in the normal lung epithelial cells.

Previous studies have shown that suppressing expression of *NDRG2* by genetic and epigenetic methods led to development of a variety of tumors including lung cancer. Li *et al* showed a decreased level of *NDRG2* in human lung cancer tissues that was associated with the invasion and tumor size. In addition, 5-years survival time of patients with low *NDRG2* expression was shorter than those with high *NDRG2* expression¹¹. Thus, *NDRG2* has become a target for cancer therapy, and numerous studies have revealed the potent effect of *NDRG2* in reducing cell viability and invasiveness of tumor cells²²⁻²⁶. Nevertheless, the role *NDRG2* overexpression in lung cancer therapy has been investigated in a few studies¹³. In the present study, human *NDRG2* gene was specifically overexpressed using Sur-P in A549 cells. Consistent with the results of a previous study^{13,27}, our findings revealed that *NDRG2* overexpression decreased viability and enhanced apoptosis of A549 cells. Several possible mechanisms can describe anti-tumor effects of *NDRG2*. In human colon carcinoma cells, it has been demonstrated that *NDRG2* overexpression arrested cell cycle in G1 phase by downregulating cyclin D expression²⁸. Similarly, Kang *et al* evaluated the effects of *NDRG2* overexpression on cell proliferation of ovarian cancer cells and showed that *NDRG2* can suppress cell viability by increasing expression of apoptosis-related proteins. In addition, the authors revealed that *NDRG2* overexpression significantly arrested cell cycle of ovarian cancer cell lines by reducing expression of cyclins²⁹. To our knowledge, the mechanisms of *NDRG2* effects on the cell viability of lung tumor cells have not been investigated so far and further studies are needed to clarify the possible role cell cycle and apoptosis proteins in this issue.

The results of the current research also showed that *NDRG2* overexpression remarkably reduced the invasion and migration of A549 cells, which was in agreement with the effects of *NDRG2* on other tumor cells²³⁻²⁵. MMPs are essential enzymes for the growth, invasion, and angiogenesis of lung tumor cells³⁰. *MMP-2* (gelatinase A) is highly expressed in lung cancer and is correlated with the invasiveness of this malignancy³¹. Findings of the current study revealed a decreased level of *MMP-2* mRNA upon *NDRG2* overexpression, indicating the possible role of this enzyme in the anti-invasive effects of *NDRG2*. This finding is line with the results of our previous study which showed the inhibitory effects of *NDRG2* on *MMP-2* enzymatic activ-

ity¹³. Similarly, *NDRG2* overexpression was shown to be associated with MMPs downregulation in renal cell carcinoma³², prostate cancer²⁴, and melanoma cell lines³³. Several mechanisms may involve in suppressing effects of *NDRG2* on MMPs expression, notably inhibition of nuclear factor kappa B activity³³ and phosphoinositide 3-kinase (PI3K)/AKT signaling pathway³⁴. Further investigations are required to determine the role these signaling pathways in the anti-metastatic effects of *NDRG2* in lung cancer.

Conclusion

The study findings suggested that Sur-P resulted in the specific overexpression of *NDRG2* in lung tumor A549 cells. The results also exhibited increased cell death and apoptosis and decreased migration and invasion of A549 cells through inhibition of *MMP-2* and *MMP-9* expression. This strategy may be considered for the treatment of NSCLC. Yet, further *in vivo* studies are required to confirm the potential efficacy of this method in lung cancer therapy.

Acknowledgement

This paper was a part of Maryam Fanian's MSc thesis and was supported by grant No. 90-5971 from the Vice-Chancellor for Research Affairs of Shiraz University of Medical Sciences, Shiraz, Iran. The authors are grateful to all staff of the Diagnostic Laboratory Sciences and Technology Research Center and Shiraz Institute for Cancer Research Center for their technical assistance in this work. They would also like to appreciate Ms. A. Keivanshekouh at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for improving the use of English in the manuscript.

References

- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71(3):209-49.
- Watanabe T, Miura T, Degawa Y, Fujita Y, Inoue M, Kawaguchi M, et al. Comparison of lung cancer cell lines representing four histopathological subtypes with gene expression profiling using quantitative real-time PCR. *Cancer Cell Int* 2010;10(1):2.
- Olaussen KA, Postel-Vinay S. Predictors of chemotherapy efficacy in non-small-cell lung cancer: a challenging landscape. *Ann Oncol* 2016;27(11):2004-16.
- Min H-Y, Lee H-Y. Mechanisms of resistance to chemotherapy in non-small cell lung cancer. *Arch Pharm Res* 2021;44(2):146-64.
- Tolozan EM, Morse MA, Lyerly HK. Gene therapy for lung cancer. *J Cell Biochem* 2006;99(1):1-22.
- Alekseenko I, Pleshkan V, Sass A, Filyukova O, Snezhkov E, Sverdlov E, editors. A universal tumor-specific promoter for cancer gene therapy. *Doklady Biochemistry and Biophysics*; 2018: Springer.
- Li D, Hu C, Li H. Survivin as a novel target protein for reducing the proliferation of cancer cells. *Biomed Rep* 2018;8(5):399-406.
- Shamsabadi FT, Eidgahi MRA, Mehrbod P, Daneshvar N, Allaudin ZN, Yamchi A, et al. Survivin, a promising gene for targeted cancer treatment. *Asian Pac J Cancer Prev* 2016;17(8):3711-9.
- Chen Y-Q, Zhao C-L, Li W. Effect of hypoxia-inducible factor-1 α on transcription of survivin in non-small cell lung cancer. *J Exp Clin Cancer Res* 2009;28(1):1-8.
- Chen Y, Wang X, Li W, Zhang H, Zhao C, Li Y, et al. Sp1 upregulates survivin expression in adenocarcinoma of lung cell line A549. *Anat Rec (Hoboken)* 2011;294(5):774-80.
- Li S-j, Wang W-y, Li B, Chen B, Zhang B, Wang X, et al. Expression of *NDRG2* in human lung cancer and its correlation with prognosis. *Med Oncol* 2013;30(1):1-8.
- Wang H, Wang W, Wang X, Cai K, Wu H, Ju Q, et al. Reduced N-Myc downstream-regulated gene 2 expression is associated with CD24 upregulation and poor prognosis in patients with lung adenocarcinoma. *Med Oncol* 2012;29(5):3162-8.
- Faraji SN, Mojtahedi Z, Ghalamfarsa G, Takhshid MA. N-myc downstream regulated gene 2 overexpression reduces matrix metalloproteinase-2 and-9 activities and cell invasion of A549 lung cancer cell line in vitro. *Iran J Basic Med Sci* 2015;18(8):773.
- Yang L, Cao Z, Li F, Post D, Van Meir E, Zhong H, et al. Tumor-specific gene expression using the survivin promoter is further increased by hypoxia. *Gene Ther* 2004;11(15):1215-23.
- Hashemi ZS, Moghadam MF, Farokhimanesh S, Rajabibazl M, Sadroddiny E. Inhibition of breast cancer metastasis by co-transfection of miR-31/193b-mimics. *Iran J Basic Med Sci* 2018;21(4):427-33.
- Hashemi ZS, Moghadam MF, Sadroddiny E. Varying miR-193b-3p expression patterns in breast cancer cell Lines indicates its potential for cancer management strategies. *International Journal of Cancer Management* 2018 Aug 31;11(8):e63540.
- Martínez-García D, Manero-Rupérez N, Quesada R, Korrodi-Gregório L, Soto-Cerrato V. Therapeutic strategies involving survivin inhibition in cancer. *Med Res Rev* 2019;39(3):887-909.
- Zhu ZB, Makhija SK, Lu B, Wang M, Kaliberova L, Liu B, et al. Transcriptional targeting of tumors with a novel tumor-specific survivin promoter. *Cancer Gene Ther* 2004;11(4):256-62.
- Bao R, Connolly DC, Murphy M, Green J, Weinstein JK, Pisarcik DA, et al. Activation of cancer-specific gene expression by the survivin promoter. *J Natl Cancer Inst* 2002;94(7):522-8.
- Mohammadi V, Behbahani AB, Rafiee GR, Hosseini SY, Zarei MA, Okhovat MA, et al. The effects of specific expression of apoptin under the control of PSES and PSA

- promoter on cell death and apoptosis of LNCaP cells. *Iran J Basic Med Sci* 2017;20(12):1354-9.
21. Darayee M, Geramizadeh B, Tabei SMB, Rezvani A, Soleimanian S, Rahimi A. Suggesting Tissue-Specific MSMB Gene Promoter as a Novel Approach for Prostate Targeted Gene Therapy. *Asian Pac J Cancer Prev* 2022; 23(6):1993-2000.
 22. Chen X-L, Lei L, Hong L-L, Ling Z-Q. Potential role of NDRG2 in reprogramming cancer metabolism and epithelial-to-mesenchymal transition. *Histol Histopathol* 2017;33(7):655-63.
 23. Golestan A, Mojtahedi Z, Ghalamfarsa G, Hamidinia M, Takhshid MA. The effects of NDRG2 overexpression on cell proliferation and invasiveness of SW48 colorectal cancer cell line. *Iran J Med Sci* 2015;40(5):430.
 24. Zarei MA, Dehbidi GR, Takhshid MA. Combination of NDRG2 overexpression, X-ray radiation and docetaxel enhances apoptosis and inhibits invasiveness properties of LNCaP cells. *Urol Oncol* 2020 Nov;38(11):849.e1-849.e9.
 25. Farokhinejad F, Behbahani AB, Dehbidi GRR, Takhshid MA. Expression and purification of TAT-NDRG2 recombinant protein and evaluation of its anti-proliferative effect on LNCaP cell line. *Protein Expr Purif* 2017;138: 25-33.
 26. Zarei MA, Takhshid M, Behbahani AB, Hosseini S, Okhovat M, Dehbidi GRR, et al. Synergistic effects of NDRG2 overexpression and radiotherapy on cell death of human prostate LNCaP cells. *J Biomed Phys Eng* 2017; 7(3):257.
 27. Tantai J, Pan X, Hu D. RNF4-mediated SUMOylation is essential for NDRG2 suppression of lung adenocarcinoma. *Oncotarget* 2016;7(18):26837-43.
 28. Kim YJ, Yoon SY, Kim JT, Choi SC, Lim JS, Kim JH, et al. NDRG2 suppresses cell proliferation through down-regulation of AP-1 activity in human colon carcinoma cells. *Int J Cancer* 2009;124(1):7-15.
 29. Kang F, Wang Y, Luo Y, Zhang Y. NDRG2 gene expression pattern in ovarian cancer and its specific roles in inhibiting cancer cell proliferation and suppressing cancer cell apoptosis. *J Ovarian Res* 2020;13(1):48.
 30. Merchant N, Nagaraju GP, Rajitha B, Lammata S, Jella KK, Buchwald ZS, et al. Matrix metalloproteinases: their functional role in lung cancer. *Carcinogenesis* 2017;38 (8):766-80.
 31. Sharma A, Mathur R, Malhotra H. Matrix metalloproteinase-2: a possible marker for non-small cell lung carcinoma. *Journal of Clinical & Diagnostic Research* 2020;14(3).
 32. Ma JJ, Kong LM, Liao CG, Jiang X, Wang Y, Bao TY. Suppression of MMP-9 activity by NDRG2 expression inhibits clear cell renal cell carcinoma invasion. *Med OncoL* 2012;29(5):3306-13.
 33. Kim A, Kim MJ, Yang Y, Kim JW, Yeom YI, Lim JS. Suppression of NF-kappaB activity by NDRG2 expression attenuates the invasive potential of highly malignant tumor cells. *Carcinogenesis* 2009;30(6):927-36.
 34. Nakahata S, Ichikawa T, Maneesaay P, Saito Y, Nagai K, Tamura T, et al. Loss of NDRG2 expression activates PI3K-AKT signalling via PTEN phosphorylation in ATLL and other cancers. *Nat Commun* 2014;5:3393.