

Microarray-based Analysis of Genes, Transcription Factors, and Epigenetic Modifications in Lung Cancer Exposed to Nitric Oxide

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Abstract. *Background/Aim:* Nitric oxide (NO) is recognized as an important biological mediator that exerts several human physiological functions. As its nature is an aqueous soluble gas that can diffuse through cells and tissues, NO can affect cell signaling, the phenotype of cancer and modify surrounding cells. The variety of effects of NO on cancer cell biology has convinced researchers to determine the defined mechanisms of these effects and how to control this mediator for a better understanding as well as for therapeutic gain. *Materials and Methods:* We used bioinformatics and pharmacological experiments to elucidate the potential regulation and underlying mechanisms of NO in non-small a lung cancer cell model. *Results:* Using microarrays, we identified a total of 151 NO-regulated genes (80 up-regulated genes, 71 down-regulated genes) with a strong statistically significant difference compared to untreated controls. Among these, the genes activated by a factor of more than five times were: DCBLD2, MGC24975, RAB40AL, PER3, RCN1, MRPL51, PTTG1, KLF5, NFIX. On the other hand, the expression of RBMS2, PDP2, RBAK, ORMDL2, GRPEL2, ZNF514, MTHFD2, POLR2D, RCBTB1, JOSD1, RPS27, GPR4 genes were significantly decreased by a factor of more than five times. Bioinformatics further revealed that

NO exposure of lung cancer cells resulted in a change in transcription factors (TFs) and epigenetic modifications (histone modification and miRNA). Interestingly, NO treatment was shown to potentiate cancer stem cell-related genes and transcription factors Oct4, Klf4, and Myc. *Conclusion:* Through this comprehensive approach, the present study illustrated the scheme of how NO affects molecular events in lung cancer cells.

Lung cancer is one of the leading causes of cancer-related deaths worldwide (1, 2). Since the aggressive phenotypes and treatment failures have been frequently observed in lung cancer patients (3-5), researchers are currently focusing on research for novel therapeutic targets towards development of more precise therapeutic options.

The impact of the cancer microenvironment in progression of the disease has been well established (6). Nitric oxide (NO), one of the most recognized substances in the microenvironment, is frequently up-regulated in certain cancers including lung cancer (7-9). NO is a short-lived gas that is endogenously produced by several cells in the human body. It is synthesized from arginine by a group of enzymes named nitric oxide synthases (NOS) (10, 11). Although it is known that a substantial amount of NO can regulate normal physiological activities such as vasodilation and neurotransmission, the dysregulation or excessive production of NO contributes to pathophysiological conditions. Indeed, high NOS expression has been detected in many cancers including breast, cervical, head and neck, central nervous system, and lung cancers (9-12). NO has been linked to cancers and has been proposed to have dual effects (13, 14). Several studies have revealed that NO promotes tumor growth and progression at low and moderate concentrations (6, 7, 9). On other hand, NO at high concentrations could exert a cancer cell killing effect (15, 16). In lung cancer, NO

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has been shown to mediate aggressive cancer phenotypes in terms of migration and invasion (17), anoikis resistance (18), chemotherapeutics drugs resistance (19), and cancer stem cell-like behaviors (20, 21). Despite the fact that the current known molecular signaling pathways of NO are insufficient to explain all its activities in cancer biology, the high-throughput technology with global database, such as microarrays and bioinformatics, would be a useful tool to provide a better understanding of the effects of NO and aid in search for novel cancer drug targets that are affected by NO. Bioinformatics has become an important tool for molecular biology studies, especially regarding interpretation of microarray data (22). Although a microarray can provide a vast amount of biological information, the direct output of microarrays cannot be used without appropriate analysis by a database analytical tool (23). Bioinformatics utilizes biological data from various validated sources and provides valuable data sets, including potential transcription factors and epigenetics modifications, suggested cellular pathways, and the ontology of gene expression (24).

Using the Affymetrix microarray, we determined the gene expression pattern in response to NO treatment. Bioinformatics analysis further described the NO-regulated genes, focusing on the transcription factors (TFs) and epigenetics modifications (histone modification and miRNA). Paying considerable attention to the molecular effects of NO on cancer cells will remove certain discrepancies in the field of knowledge and facilitate a great in-depth understanding of how this important biological regulator controls cancer cells.

Materials and Methods

Cells and reagents. Human non-small cell lung cancer cell line, H460, was obtained from the American Type Culture Collection ATCC (Manassas, VA, USA). The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/ml of penicillin and streptomycin (GibCo) in 5% CO₂-humidified incubator under 37°C. Cells were routinely sub-cultured at 70% confluent density using a 0.25% trypsin solution with 0.53 mM EDTA. Nitric oxide (NO) donor, dipropylentriamine (DPTA) NONOate, was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Previous studies showed that DPTA (NO donor) was relatively non-toxic to non-small cell lung cancer cell lines (17, 21, 25), and the non-toxic concentration for H460 cells were 0-50 μM. In this study, 10×10⁴ cells of H460 were seeded overnight and treated with 40 μM of DPTA for 5 days (the freshly prepared DPTA was replace every day). Treated and untreated control cells were subjected to gene expression analysis.

Microarray analysis. After specific treatment, the cells were collected. The mRNA of two independent experiments were harvested and cDNA was prepared according to the Affymetrix's protocol. In brief, 10 μg of total RNA was reverse transcribed using SuperScript II RT (Invitrogen, CA, USA) and T7-(dT)24 primer. After double-strand cDNAs were created, the cDNAs were purified

and precipitated by phenol-chloroform extraction/ethanol precipitation. The cDNAs were subjected to synthesis biotin-labelled cRNA and quantified by a NanoDrop-1000. Fifteen micrograms of cRNA were used to make 300 μl of hybridization cocktail. A total of 225 μl of the cocktail were used for target hybridization on the gene chip as Affymetrix's procedure. After washing, slides were scanned in an Agilent GeneArray Scannerc (Agilent, CA, USA).

Biological process analysis. The 151 NO-regulated genes were subjected to String (<https://string-db.org/>) to predict cell biological processes mediated by NO. This result was analyzed by date 20th September 2019.

Prediction of potential transcription factors. The 151 NO-mediated genes were analyzed by Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>) for determining the transcription factors that could control the expression of these genes. We analyzed the NO-mediated genes with Enrichr using gene set libraries as follows: ChIP enrichment analysis (ChEA) (<https://genome.ucsc.edu/ENCODE/>), Enrichr for Protein-Protein Interactions (PPIs) (<http://amp.pharm.mssm.edu/Enrichr/>), encyclopedia of DNA elements (ENCODE) TF ChIP-seq 2015 (<https://genome.ucsc.edu/ENCODE/>), TRANSFAC and JASPAR PWMs (<http://genexplain.com/transfac/> and <http://jaspar.genereg.net/>), Enrichr Submission TF-Gene co-occurrence (<http://amp.pharm.mssm.edu/Enrichr/>), ENCODE and ChEA consensus TFs from ChIP-X (<https://genome.ucsc.edu/ENCODE/>), and ARCHS4 TFs (<https://amp.pharm.mssm.edu/archs4/>). The visualization of transcription factors hub networks and the calculation of p-value, odds ratio (OR), and combined scores were constructed by Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>). The results were analyzed by date 20th September 2019.

Screening of potential epigenetic modifications. Histone modifications and epigenetic alterations mediated by NO were analyzed by Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>). We investigated the histone modification and potential miRNA with Enrichr using gene set libraries as follows: ENCODE histone modifications 2015 (<https://genome.ucsc.edu/ENCODE/>), TargetScan microRNA2017 (http://www.targetscan.org/vert_72/), and miTarBase2017 (<http://miRTarBase.mbc.nctu.edu.tw>). The visualization of histone modification hub networks and the calculation of p-value, OR, and combined score were constructed by Enrichr. The results were analyzed by date 20th September 2019.

Statistical analysis. The mRNA expression levels of two independent experiments were analyzed by the two-tailed Student's *t*-test to compare mean expression levels and identify NO-specific genes. All statistical analysis processes were performed by Connection Up- and Down-Regulation Expression of Microarrays (CU-DREAM) software (26). Statistical significance was set at $p < 0.05$. The 151 NO-mediated genes that were strongly statistically altered between control and treatment groups ($p < 0.01$) were subjected to examine for potential transcription factors, histone modification, and miRNA by bioinformatic databases.

Results

Identification of NO-affected genes in non-small lung cancer cells. Two independent sets of NSCLC H460 cells were continuously exposed to NO donor (DPTA NONOate) at

Table I. Genes that were significantly altered by nitric oxide treatment.

Effect of NO on genes expression	Gene names
Up-regulated genes expression	<i>PER3, MRTO4, FAM167B, ZNF362, CELSR2, S100A1, SNAPIN, SNRPE, SNIP1, CASQ2, OR6P1, GCSH, RCN1, RHOG, OR5B17, C11orf75, THY1, GPD1, RPS26, MYL6B, INHBC, TRHDE, TNFRSF1A, MRPL51, GALNT6, IL17D, KLF5, LAMP1, OR10G2, CMTM2, G6PC, MYH3, UBE2S, AATK, COLEC12, HMG20B, NFIX, CD3EAP, MGC24975, FBXL12, LGI4, FLJ32063, FBXO48, KRTAP19-8, C21orf77, DSCR4, ICOSLG, PDXP, ARGFX, COL29A1, C3orf75, DCBLD2, STAG1, HTN1, CDS1, HSPA4L, PPBP, CENPE, FLJ37543, PTTG1, TAF7, FTSJD2, RFPL4B, PLN, FAM164B, MASI, PRRT1, VWC2, EPHB6, CRIM2, NUDT18, SFRP1, SURF6, RAB40AL, HPRT1, SAGE1, KLHL34, GUCY2F, MAGEA1, RBMY1B</i>
Down-regulated genes expression	<i>RPL11, RPS27, KCTD3, SDCCAG8, ARL5B, PSTK, PRKCQ, PLAC1L, ASB9, CEP164, ORMDL2, RBMS2, C12orf45, KNTC1, BRI3BP, NRIP2, WBP11, CCDC59, RCBTB1, SERPINA3, CCDC88C, LOC646358, NDUFB10, OR2C1, PDP2, POLR3K, RPS2, ICT1, KIAA1328, PIK3C3, YIPF2, GPR4, MTHFD2, ARHGAP15, ZNF514, ERCC3, POLR2D, BAZ2B, INO80D, CHMP4B, CTA-221G9.4, GRAP2, JOSD1, GFM1, SENP2, FYCO1, BCHE, KCNMB3, UGT2B7, GRPEL2, HMGXB3, ZNF354B, DDX41, IRAK1BP1, FAM46A, SRrp35, RBAK, LOC255374, PRSS1, OR6V1, CLDN23, HSF1, LONRF1, KCTD9, MRPS2, MAN1B1, RLN1, ZNF782, PLP2, EFNBI, SASH3</i>

non-cytotoxic concentration (40 μ M) for 5 days. NO-treated cells and their non-treated counterparts were subjected to cDNA preparation and analyzed for gene expression by microarrays, as described in Materials and Methods. After using the CU-DREAM program to analyze the raw data, the 151 gene lists with a strong significant difference ($p < 0.01$) were selected for the next interpretation, as listed in Table I.

Analysis for nitric oxide-mediated biological process alteration. The statistically significantly differentially expressed 151 NO-mediated genes were subjected to search tool for the retrieval of interacting genes/proteins (String) software for determination NO-altered biological processes. The results from the String analysis showed various cellular biological processes altered by NO exposure (data not shown). We analyzed the effects of NO on the biological processes that would usually be changed in cancer such as gene expression control (52/151 genes), ribonucleoprotein complex biogenesis (11/151 genes), regulation of translation (14/151 genes), protein targeting to endoplasmic reticulum (ER) (10/151 genes), and cellular metabolic processes (90/151 genes). Figure 1 shows protein–protein association by line-color and shows the function of proteins in each biological process by node-color. The biological process that was mostly altered by NO exposure was the cellular metabolic process (90/151 genes), represented by the green node.

The second ranking process was the gene expression process (52/151 genes) which is shown by the blue node. Interestingly, the NO-regulated genes with a high degree of connection lines have multitudinous functions in controlling of biological processes, as represented by multicolor node network in the center of diagram. The other network group of input genes was mainly involved in the regulation of

cellular metabolism, gene expression and RNA processing through controlling ribonucleoprotein complex biogenesis. The node proteins of this network group were POLR2D, SNRPE, and POLR3K.

Screening for potential transcription factors. The 151 genes with a significant difference were used to predict the potential transcription factors using the web-based bioinformatics tool, Enrichr. The results from this analysis showed the top ten potential transcription factors together with their p -value, OR, combined score, and the target genes of the transcription factor that matched with the input genes. The potential transcription factors were arranged by a combined score. The results from certain gene set libraries were recorded as follows:

ChEA 2016. Data from the ChEA 2016 gene set database showed that many potential transcription factors were responsible for NO-mediated gene alteration. For a better understand, the top ten transcription factors with high combined scores are shown in Table II, namely; STAT3, ZNF652, ETS1, MYC, TFAP2A, PRDM16, JARID1A, OCT4, SALL4, and Sox2. The networks of hub transcription factors arranged by p -value, namely KLF4, ETS1, E2A, OCT4, MYC, MAF, TOP2B, SALL4, TFAP2A, and JARID1A are shown in Figure 2A. From Figure 2(A) the hub with the highest number of linkage was OCT4.

ENCODE transcription factor (TF)-ChIP-sequences 2015. The top ten transcription factors ranked by combined score using ENCODE TF ChIP-seq 2015 gene set library were CUX1, FOXM1, RFX5, RAD21, SMC3, EBF1, TAF1, ELK1, CREB1, and EBF1, as shown in Table III. However, transcription factor hub analysis by p -value demonstrated

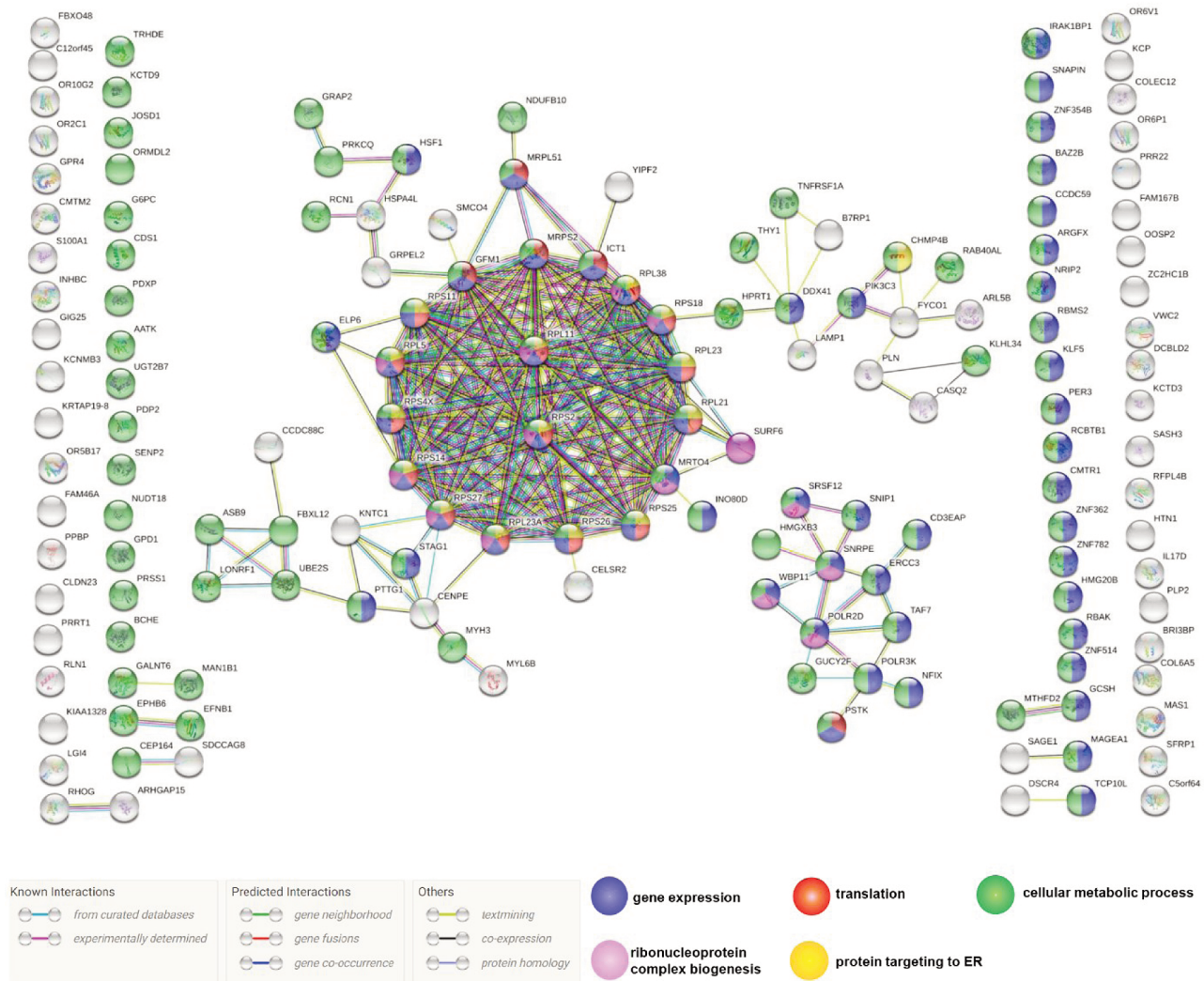


Figure 1. Pathway analysis by String. The 151 NO-regulated genes were subjected to String for analyzing the pathways regulated by NO.

that TRIM28 was the factor with the highest number of connection lines with other transcription factors (Figure 2B).

Protein-protein interaction (PPI) network. The top ten enriched transcription factors for the target genes of NO-treated factors from PPI gene set analysis ranged by combined score were: SIX5, ILF2, MEF2A, SMAD2, ZBTB7B, NR2F2, TBP, ILF3, RARA, and USF as shown in Table IV. For the network transcription factor interaction, the highest-ranking linked hub was TBP, as shown in Figure 2C.

Transcription factor database (TRANSFAC) and JASPAR position-specific weight matrices (PWMs). Analysis of the NO-mediated 151 genes alteration by TRANSFAC and JASPAR PWMs gene set libraries gave the top ten factors ranked by combined score as ESR2, RARA, JDP2, NR1H2,

FOXA1, SND1, FOXO3A, Myb, IRF1, and ELK1, as listed in Table V. Moreover, the database also provided the transcription factors hub linkage displayed in Figure 2D. This result showed that RARA has the highest number of network lines compared to other transcription factors.

Enrichr submission transcription factor-gene co-occurrence. This analysis gave the top ten potential transcription factors ranked by combined score, as listed in Table VI (ZNF593, CPSF4, ZNF511, BOLA3, HMGB3, PRMT3, THAP11, YEATS4, ZMAT2, and ZNF121).

ENCODE and ChEA consensus TFs from ChIP-X. The results showed the ranking of transcription factors by combined score as CBX3, FOXM1, ATF2, SALI4, MAX, KAT2A, IRF3, POU5F1, NR2C2, and PPARG (Table VII).

Table II. The ChEA 2016 top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
STAT3_22323479_ChIP-Seq_MACROPHAGE_Mouse	0.033338	6.97	23.71	CCDC59; MRPS2
ZNF652_21678463_ChIP-ChIP_ZR75-1_Human	0.05303	3.55	10.42	CENPE; STAG1; TNFRSF1A
ETS1_20019798_ChIP-Seq_JURKAT_Human	0.004653	1.81	9.74	ZNF782; GFM1; SNAPIN; NDUFB10; RPL11; CCDC59; FBXO48; MYL6B; DDX41; RPS26; FYCO1; RPS27; LAMP1; MRTO4; SNIP1; CHMP4B; C3ORF75; SURF6; SNRPE; PIK3C3; SDCCAG8; ZNF354B
MYC_18940864_ChIP-ChIP_HL60_Human	0.011105	2.13	9.59	CDS1; KCTD9; RPS27; GRPEL2; CLDN23; POLR2D; FAM46A; CD3EAP; RLN1; BRI3BP; MGC24975; FBXL12
TFAP2A_17053090_ChIP-ChIP_MCF-7_Human	0.004367	1.74	9.45	COLEC12; SERPINA3; OR2C1; NFIX; CEP164; AATK; GUCY2F; RCBTB1; EFNB1; GRAP2; NRIP2; MAGEA1; OR5B17; LGI4; WBP11; JOSD1; TNFRSF1A; MRPL51; MYH3; UBE2S; ASB9; CHMP4B; HPRT1; CMTM2; RBMS2
PRDM16_22522345_ChIP-ChIP_PALATE_MESENCHYMAL_Mouse	0.065124	3.26	8.90	GALNT6; LONRF1; TRHDE
JARID1A_20064375_ChIP-Seq_MESCs_Mouse	0.006295	1.65	8.35	GFM1; NDUFB10; MYL6B; DDX41; LAMP1; ICT1; ORMDL2; GRPEL2; SNIP1; SURF6; RPS2; YIPF2; CCDC59; MRPS2; KCTD3; WBP11; SENP2; FBXL12; KCTD9; ERCC3; MRTO4; GPD1; TAF7; PIK3C3; MAN1B1; RBAK; POLR3K
OCT4_19829295_ChIP-Seq_ESCs_Human	0.008089	1.66	7.98	SASH3; COLEC12; GFM1; HSPA4L; DDX41; LAMP1; GRAP2; MAGEA1; C3ORF75; KNTC1; HMGXB3; OR5B17; MRPS2; JOSD1; FBXL12; RPS26; STAG1; MRPL51; MYH3; PER3; MTHFD2; UBE2S; CASQ2; SNRPE; RAB40AL
SALL4_18804426_ChIP-ChIP_XEN_Mouse	0.020018	1.85	7.22	S100A1; LGI4; GPR4; RCBTB1; KCTD9; EFNB1; STAG1; RCN1; PLN; KLF5; CASQ2; CHMP4B; TAF7; RBMS2
SOX2_18555785_ChIP-Seq_MESCs_Mouse	0.035175	2.13	7.14	RPS26; STAG1; GRAP2; GRPEL2; CASQ2; KLHL34; PRKCQ; FBXL12

OR: Odds ratio.

ARCHS4 TFs. Analysis using the gene set from ARCHS4 TFs library showed the top ten transcription factors ranked by combined score were KLF15, ZNF528, MYF6, ZBED1, PRDM15, GPR155, SOX10, POU6F2, ELK3, and NPAS1 as presented in Table VIII.

Screening for potential miRNA and histone modifications. The 151 NO-mediated genes with a strong difference between treated and untreated groups were used to predict the epigenetic alteration effects from NO exposure using the web-based bioinformatic tool, Enrichr, as described in Material and Methods. The results from these analysis tools showed the top ten suggested histone modifications and potential miRNA together with p-value, OR, combined score, and target gene of the transcription factor that matched with the input genes. The results were arranged by their combined score.

ENCODE histone modifications 2015. As gene alterations may occur by the modification of certain histone proteins, the activity of NO might rely on this cellular process. To evaluate the potential histone modifier effect of NO, 151 genes mediated by NO were analyzed using ENCODE histone modification database version 2015. The results are shown in Table IX, revealing that the top ten ranking according to combined score were: H3K79me3, H3K9me1, H3K79me2, H2AFZ, H3K36me3, H3K27ac, K4K20me1, H3K9ac, H3K79me2, and H3K36me3. The histone modification network analysis was conducted, and it was found that H3K36me3 was the hub histone modification with the highest connectivity degree (Figure 3).

TargetScan microRNA2017. miRNA is the non-coding RNA that controls the expression of coding mRNA by regulating its half-life. The TargetScan microRNA database version 2017 was used for determining the potential miRNAs altered

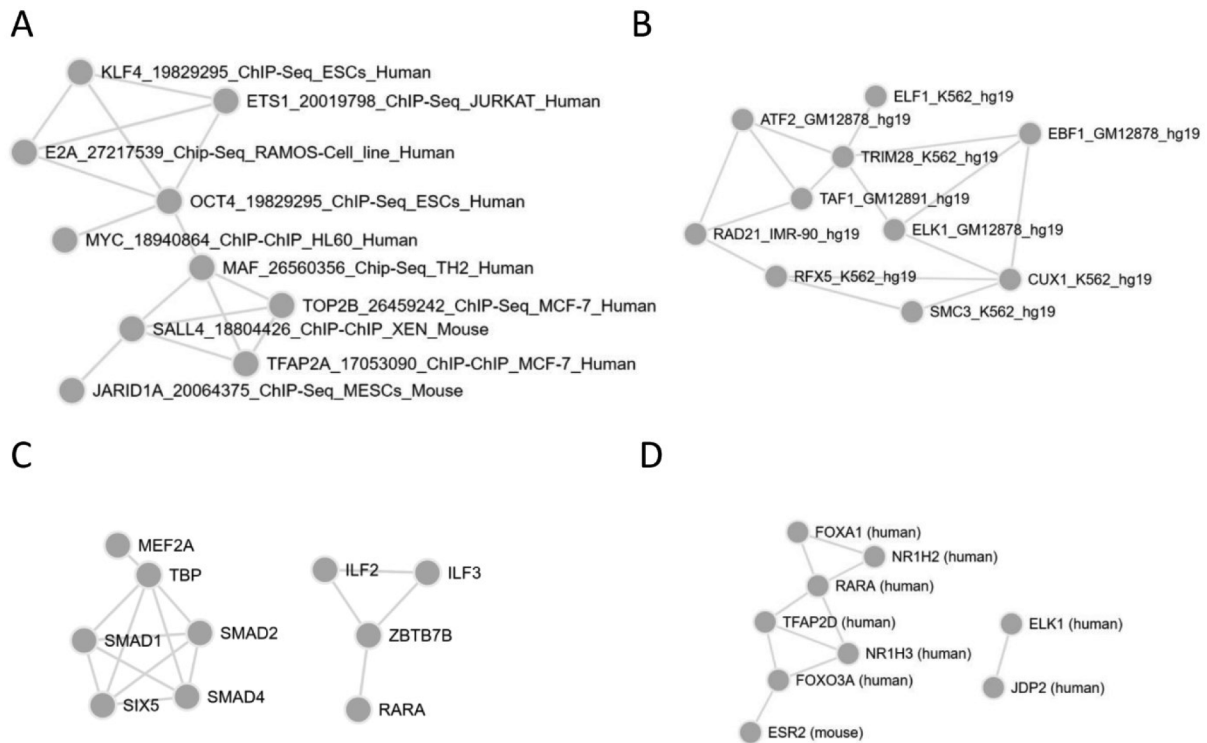


Figure 2. Potential transcription factor network. (A) Network from ChEA2016. (B) Network from ENCODE TF ChIP-seq 2015. (C) Network from Transcription Factor PPI. (D) Network from TRANSFAC and JASPAR PWMs.

by NO treatment (Table X). The analysis revealed hsa-miR-4749-5p, hsa-miR-4706, hsa-miR-615-3p, mmu-miR-3072, hsa-miR-28-3p, mmu-miR-187, mmu-miR-695, hsa-miR-4781-5p, hsa-miR-572, and mmu-miR-1947 as the top ten results.

miTarBase2017. The results from Enrichr using the miTarBase version 2017 data library revealed the suggested miRNA ranked by combined score as the following: mmu-miR-292a-3p, mmu-miR-290a-3p, mmu-miR-467a-5p, hsa-miR-3648, mmu-miR-465c-3p, mmu-miR-465b-3p, mmu-miR-465a-3p, hsa-miR-6761-3p, mmu-miR-3086-5p, and hsa-miR-4313 (Table XI).

Discussion

Lung cancer is by far the leading cause of cancer-related deaths worldwide. The key lung cancer pathological types are small cell lung carcinoma (~15%) and non-small cell lung carcinoma (NSCLC) (~85%), with the later types comprising lung squamous cell carcinoma (~40%), lung adenocarcinoma (~40%) and lung large-cell carcinoma (~10%) (2). As NSCLC is a major type of lung cancer, the mechanism of treatment failure in this type of cancer is very

interesting for researchers. The causes of drug resistance in lung cancer include cellular intrinsic mechanisms that drive the alterations of drug transporters, and the increase and activation of pro-survival and anti-apoptotic proteins (3-5, 27, 28). In addition, recent studies have highlighted the vital roles of cancer environmental factors.

One important micro-environmental factor is nitric oxide (NO). NO is a relatively short-lived gas generated in the human body by the activity of nitric oxide synthase (NOS) enzymes and has been long known to act as a signaling molecule in the regulation of several physiological events (11). On the other hand, the abnormal as well as excessive production of NO can be implicated in many diseases including cancer (9). It was shown that near tumors, NO is highly produced by the immune cells (10). In addition, cancer cells themselves have a capability to produce NO at a high level. The expression of NOS has been reported in many cancers, including breast, cervical, head and neck, and lung cancer (29), and the presence of NO in the cancer area has been linked to the aggressiveness of the diseases (12). The expression of inducible nitric oxide synthase (iNOS) in cancer cells as well as the expression of NOS in the environment of cancers have been shown to predict poor survival (30). Molecular explanations on how NO regulates chemotherapeutic resistance as well as attenuates cancer cell

Table III. The ENCODE TF ChIP-seq 2015 top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
CUX1_K562_hg19	0.002042	1.79	11.08	<i>GFM1; INO80D; NDUFB10; CEP164; FBXO48; BAZ2B; FYCO1; DSCR4; ICT1; C3ORF75; HMGXB3; YIPF2; S100A1; CD3EAP; MRPS2; FTSJD2; WBP11; TNFRSF1A; FBXL12; RPS26; CENPE; IRAK1BP1; SAGE1; TAF7; SNRPE; SDCCAG8; ZNF354B</i>
FOXM1_MCF-7_hg19	0.038685	3.15	10.26	<i>KCTD9; SERPINA3; PTTG1; UBE2S</i>
RFX5_K562_hg19	0.00415	1.72	9.44	<i>GFM1; NDUFB10; CEP164; RPL11; KIAA1328; PTTG1; GRPEL2; C3ORF75; SURF6; RPS2; HMGXB3; SNAPIN; YIPF2; MRPS2; FTSJD2; SENP2; FBXL12; RPS26; CENPE; MRPL51; KLF5; MTHFD2; MRTO4; SNRPE; MAN1B1; SDCCAG8</i>
RAD21_IMR-90_hg19	0.00415	1.72	9.44	<i>GFM1; C11ORF75; INO80D; NDUFB10; HSPA4L; RPL11; PDXP; EFN1; ARL5B; KIAA1328; ICT1; NRIP2; POLR2D; C3ORF75; ZNF362; SNAPIN; FAM46A; MRPS2; FTSJD2; RPS26; SFRP1; MRTO4; SNRPE; MAN1B1; IL17D; ZNF354B</i>
SMC3_K562_hg19	0.00415	1.72	9.44	<i>GFM1; INO80D; NDUFB10; HSPA4L; DDX41; DSCR4; LAMP1; ICT1; GRPEL2; SNIP1; NUDT18; C3ORF75; SURF6; RPS2; YIPF2; CCDC59; MRPS2; FTSJD2; RPS26; SAGE1; MRTO4; PRKCQ; SNRPE; PIK3C3; MAN1B1; POLR3K</i>
ELF1_K562_hg19	0.010994	1.80	8.13	<i>GFM1; NDUFB10; HSPA4L; CEP164; MRPS2; MYL6B; C12ORF45; RPS27; MRPL51; PDP2; PTTG1; UBE2S; ORMDL2; SURF6; SNRPE; RBAK; SDCCAG8; ZNF354B</i>
TAF1_GM12891_hg19	0.007749	1.66	8.07	<i>SASH3; MYL6B; DDX41; FYCO1; PTTG1; ORMDL2; GRPEL2; HSF1; NUDT18; C3ORF75; SURF6; RPS2; HMGXB3; ICOSLG; SNAPIN; MRPS2; FTSJD2; C12ORF45; STAG1; ERCC3; MTHFD2; MRTO4; SNRPE; MAN1B1; RBAK</i>
ELK1_GM12878_hg19	0.008089	1.66	7.98	<i>GFM1; INO80D; NDUFB10; HSPA4L; CEP164; RCBTB1; EFN1; KIAA1328; POLR2D; SNIP1; KNTC1; SNAPIN; YIPF2; FTSJD2; SENP2; TNFRSF1A; RPS26; C12ORF45; ERCC3; SNRPE; PIK3C3; MAN1B1; RBAK; POLR3K; ZNF354B</i>
CREB1_GM12878_hg19	0.018309	1.99	7.95	<i>RPS26; CENPE; RPS27; SNIP1; NUDT18; CHMP4B; PIK3C3; RPS2; WBP11; LONRF1; SENP2; RBMS2</i>
EBF1_GM12878_hg19	0.005999	1.54	7.88	<i>SASH3; NDUFB10; RPL11; PDXP; MYL6B; FYCO1; ARL5B; KIAA1328; LAMP1; GRPEL2; KCNMB3; SNIP1; C3ORF75; RPS2; HMGXB3; YIPF2; RHOG; MRPS2; FTSJD2; SENP2; FBXL12; RPS26; KCTD9; IRAK1BP1; STAG1; RPS27; MRPL51; PDP2; SNRPE; PIK3C3; PLP2; RBAK; POLR3K; SDCCAG8</i>

OR: Odds ratio.

response to immune cell-mediated death have been suggested by many researchers (11, 31, 32). NO was shown to control cancer cell response by directly interacting with the key proteins through S-nitrosylation which alter the stability and function of the target protein. S-nitrosylation at the cysteine of

the Bcl-2 protein was demonstrated to be a possible cause of cisplatin resistance in NSCLC (19). Taken together, determination of the molecular mechanism underlying the controls of NO on lung cancer cells could lead to a better understanding and development of new therapeutic targets.

Table IV. The transcription factor PPIs top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
SIX5	0.04446	22.08	68.72	<i>ERCC3</i>
ILF2	0.00277	4.32	25.44	<i>RPS26; RCN1; LAMP1; ICT1; RPL11; RPS2</i>
MEF2A	0.03174	7.16	24.70	<i>NFIX; PRKCQ</i>
SMAD2	0.00261	3.10	18.47	<i>EPHB6; STAG1; SNAPIN; KLF5; ERCC3; S100A1; SNIP1; SURF6; SENP2</i>
ZBTB7B	0.12757	7.36	15.15	<i>GRAP2</i>
NR2F2	0.15365	6.02	11.28	<i>FAM46A</i>
TBP	0.03324	3.31	11.27	<i>KLF5; HSF1; CD3EAP; TAF7</i>
ILF3	0.02544	2.68	9.82	<i>RPS26; RCN1; LAMP1; RPL11; RPS2; PLP2</i>
RARA	0.04384	3.03	9.47	<i>KLF5; NRIP2; MRTO4; BAZ2B</i>
USF2	0.17269	5.30	9.30	<i>HSPA4L</i>

OR: Odds ratio.

Table V. The TRANSFAC and JASPAR PWMs top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
ESR2 (mouse)	0.056767	5.19	14.90	<i>NUDT18; PIK3C3</i>
RARA (human)	0.003698	1.92	10.76	<i>YIPF2; HSPA4L; CEP164; MYL6B; FTSJD2; JOSD1; GCSH; RPS26; SFRP1; RCN1; RPS27; KIAA1328; HSF1; SURF6; HMG20B; SNRPE; RLN1; MAN1B1; PLP2; RBMS2</i>
JDP2 (human)	0.004029	1.87	10.31	<i>LG14; FTSJD2; BAZ2B; WBP11; GUCY2F; CENPE; ARGFX; STAG1; RFPL4B; MRPL51; PLN; PDP2; ICT1; ASB9; PRKCQ; SNRPE; RLN1; RPS2; CMTM2; POLR3K; RBMY1B</i>
NR1H2 (human)	0.029206	2.21	7.82	<i>KIAA1328; YIPF2; RPL11; HSF1; HMG20B; SNRPE; MYL6B; RLN1</i>
FOXA1 (human)	0.026529	1.64	5.94	<i>G6PC; RPL11; PDXP; KLHL34; KCTD3; SENP2; INHBC; RPS26; RPS27; GRPEL2; CLDN23; KCNMB3; ZNF514; SNRPE; HPRT1; POLR3K; RAB40AL; SDCCAG8</i>
SND1 (mouse)	0.148033	2.94	5.62	<i>TRHDE; TNFRSF1A</i>
FOXO3A (human)	0.044372	1.79	5.58	<i>FAM167B; IRAK1BP1; G6PC; NFIX; CLDN23; RPL11; KCNMB3; KLHL34; SNRPE; POLR3K; SDCCAG8</i>
Myb (mouse)	0.028672	1.53	5.44	<i>CDS1; S100A1; CEP164; DDX41; ARHGAP15; SENP2; TNFRSF1A; DCBLD2; GCSH; KCTD9; FAM167B; RFPL4B; RPS27; MRPL51; MYH3; ORMDL2; VWC2; SNIP1; NUDT18; MAN1B1; POLR3K; SDCCAG8</i>
IRF1 (human)	0.2561	3.40	4.63	<i>RFPL4B</i>
ELK1 (human)	0.063785	1.49	4.10	<i>C11ORF75; INO80D; LG14; CEP164; CD3EAP; MYL6B; RPS26; FLJ32063; RPS27; MRPL51; KLF5; SAGE1; GRAP2; CCDC88C; TAF7; RLN1; RBMY1B</i>

OR: Odds ratio.

Focusing on novel molecular-based insights, it has become widely accepted that tumors are composed of heterogeneous cells with a variety of characteristics that make the cells respond differently to treatment (33). Besides, after chemotherapeutic management, lung cancer often develops

resistant clones. It is now accepted that cancer heterogeneity is mainly governed by a small special subpopulation of cancer cells with high self-renewal capacity, termed cancer stem cells (CSCs). CSCs have the ability to initiate tumor through their self-renewing and multi-lineage differentiation

Table VI. The Enrichr Submission TF-Gene co-occurrence top ten of enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
ZNF593	0.00046	3.99	30.65	RPS26; PTTG1; NDUFB10; MTHFD2; UBE2S; MRTO4; RPL11; CD3EAP; RPS2
CPSF4	0.00200	3.54	22.03	CENPE; MTHFD2; MRTO4; POLR2D; CD3EAP; HMG20B; MRPS2; RPS2
ZNF511	0.00200	3.54	22.03	PTTG1; NDUFB10; MTHFD2; UBE2S; MRTO4; CD3EAP; RPS2; POLR3K
BOLA3	0.00772	3.10	15.08	RPS26; MRPL51; PTTG1; NDUFB10; MTHFD2; CD3EAP; RPS2
HMGB3	0.00772	3.10	15.08	CENPE; PTTG1; MTHFD2; UBE2S; CD3EAP; KNTC1; HPRT1
PRMT3	0.00772	3.10	15.08	PTTG1; MTHFD2; MRTO4; POLR2D; CD3EAP; KNTC1; HPRT1
THAP11	0.00772	3.10	15.08	NDUFB10; MTHFD2; RPL11; POLR2D; CD3EAP; MRPS2; RPS2
YEATS4	0.00772	3.10	15.08	CENPE; PTTG1; MTHFD2; CD3EAP; KNTC1; SNRPE; HPRT1
ZMAT2	0.00772	3.10	15.08	RPS26; NDUFB10; RPL11; CD3EAP; CHMP4B; SNRPE; RPS2
ZNF121	0.00772	3.10	15.08	CENPE; ARL5B; MTHFD2; GRPEL2; POLR2D; CD3EAP; KNTC1

OR: Odds ratio.

Table VII. The ENCODE and ChEA consensus TFs from ChiP-X top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
CBX3_ENCODE	0.023455	4.91	18.41	GFM1; LAMP1; CCDC59
FOXM1_ENCODE	0.035281	4.18	13.99	KCTD9; PTTG1; UBE2S
ATF2_ENCODE	0.00736	1.53	7.53	INOS80; NDUFB10; RPL11; FBXO48; BAZ2B; LAMP1; ICT1; ORMDL2; GRPEL2; SNIP1; NUDT18; SURF6; KNTC1; RPS2; HMGXB3; PSTK; YIPF2; CCDC59; CD3EAP; JOSD1; SENP2; RPS26; CENPE; IRAK1BP1; STAG1; RPS27; MRPL51; MTHFD2; UBE2S; CCDC88C; TAF7; SNRPE; RBMS2
SALL4_CHEA	0.05283	2.24	6.58	STAG1; RCN1; PLN; KLF5; CASQ2; PRKCQ
MAX_ENCODE	0.022343	1.53	5.83	GFM1; RPL11; RHOG; CD3EAP; MRPS2; PDXP; DDX41; SENP2; RCBTB1; GCSH; MRPL51; PER3; KLF5; LAMP1; MTHFD2; UBE2S; MRTO4; CCDC88C; HSF1; SURF6; SNRPE; MAN1B1; RPS2; POLR3K
KAT2A_ENCODE	0.148033	2.94	5.62	RPL11; RPS2
IRF3_ENCODE	0.064431	1.80	4.93	KCTD9; PDP2; KLF5; MTHFD2; ORMDL2; CEP164; PDXP; KNTC1; WBP11
POU5F1_CHEA	0.135689	2.03	4.05	KCTD9; SFRP1; STAG1; GRAP2
NR2C2_ENCODE	0.126159	1.89	3.92	RPS26; GFM1; CCDC59; CHMP4B; SURF6
PPARG_CHEA	0.11117	1.73	3.81	GALNT6; STAG1; NDUFB10; GPD1; RBAK; SENP2; SDCCAG8

OR: Odds ratio.

(34, 35). Regarding lung cancer, many studies have reported the presence of CSCs in NSCLC and associated them to drug resistance, metastasis, and cancer relapse (36). Moreover, several pieces of evidences have shown that NO can promote

the aggressive behavior of cancer for which CSCs are responsible (7, 17, 18, 20, 21, 25).

Herein, we focused on illustrating the molecular profile of the NSCLC exposed to a non-toxic concentration of NO that

Table VIII. The ARCHS4 TFs top ten enriched transcription factors affected by DPTA NONOate.

Transcription factors	p-Value	OR	Combined score	Targeted genes from input genes
KLF15_human_tf_ARCHS4_coexpression	0.001997	3.54	22.03	<i>FYCO1; G6PC; NFIX; S100A1; GPD1; IL17D; INHBC; UGT2B7</i>
ZNF528_human_tf_ARCHS4_coexpression	0.001997	3.54	22.03	<i>C12ORF45; MASI; KIAA1328; PIK3C3; GPR4; BAZ2B; HTN1; SDCCAG8</i>
MYF6_human_tf_ARCHS4_coexpression	0.007723	3.10	15.08	<i>FYCO1; PLN; S100A1; CASQ2; KLHL34; PRKCQ; IL17D</i>
ZBED1_human_tf_ARCHS4_coexpression	0.007723	3.10	15.08	<i>SASH3; FYCO1; LAMP1; HSF1; CHMP4B; HMG20B; TNFRSF1A</i>
PRDM15_human_tf_ARCHS4_coexpression	0.007723	3.10	15.08	<i>C12ORF45; KIAA1328; PDP2; CCDC88C; BRI3BP; ICOSLG; HTN1</i>
GPR155_human_tf_ARCHS4_coexpression	0.007723	3.10	15.08	<i>C12ORF45; MASI; KIAA1328; FBXO48; PIK3C3; BAZ2B; HTN1</i>
SOX10_human_tf_ARCHS4_coexpression	0.026176	2.66	9.68	<i>FAM167B; NFIX; S100A1; LGI4; AATK; HMG20B</i>
POU6F2_human_tf_ARCHS4_coexpression	0.026176	2.66	9.68	<i>C12ORF45; MASI; KIAA1328; VWC2; BAZ2B; HTN1</i>
ELK3_human_tf_ARCHS4_coexpression	0.026176	2.66	9.68	<i>KCTD9; GRAP2; PPBP; PLP2; RBMS2; TNFRSF1A</i>
NPAS1_human_tf_ARCHS4_coexpression	0.026176	2.66	9.68	<i>NDUFB10; UBE2S; MRPS2; PDXP; RPS2; IL17D</i>

OR: Odds ratio.

may mimic the phenomenon in lung cancer environments. The mRNAs of NO-treated cells were analyzed with microarrays and bioinformatics approaches to identify potential transcription factors and epigenetics modifiers in comparison to those of un-treated cells. Our results showed that several transcription factors identified as molecular targets of NO play a role in the formation and maintenance of CSC (20) and the aggressive behaviors related with CSCs (17, 18, 25). These results, along with cell-based analysis, revealed that NO promotes the dedifferentiation of lung cancer by increasing the CSC transcription factors and proteins (21).

Since induced pluripotent stem cells (iPS) are originally generated from murine fibroblasts, which involve transfection by four transcription factors (yamanaka factors); namely Sox2, Oct3/4, c-Myc, and Klf4 (37), these transcription factors have been extensively examined for their role in CSCs (38). Our results predicted that the four Yamanaka factors (Oct4, Sox2, Myc, and Klf4) were potential transcription factors affected by NO. Octamer binding transcription (Oct) is a subclass of the POU transcription factor family proteins, which bind a specific eight-base-pair on DNA sequence [ATGC(A/T)AAT] (octamer motif) and control the transcriptional processes of certain genes. Oct4 acts as a core protein to facilitate the other stemness related proteins, Sox2, and nanog, to form a transcription factor complex and promote the transcriptional process of stemness genes (39, 40). There is strong evidence indicating Oct4 has a CSC formation capacity and its malignancy phenotype has an enhancing effect (41-44). SYR-related HMG-box (Sox) is a transcription factor binding to ATTGTT or a related sequence on the HMG domain and it regulates both the differentiation and dedifferentiation process

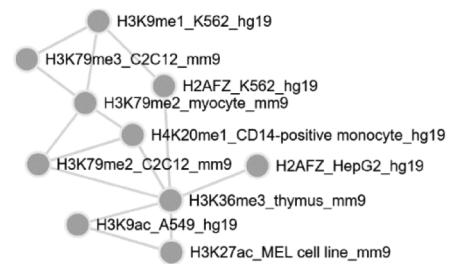


Figure 3. Potential epigenetics network. Histone modification network from ENCODE histone modifications 2015.

of cells. The function of the Sox protein on differentiation processes of the cell depends on its transcription protein partner (45-47). Sox2 has been observed to have dedifferentiation activity both in normal and cancer cells. A high protein expression of Sox2 and Oct4 is related to poor prognosis and aggressiveness of the disease (48, 49). MYC is a protein family of a group of basic-helix-loop-leucine zipper transcription factors that regulate cell proliferation, growth, metabolism, cell-cycle progression, and differentiation. This family consists with three forms, c-Myc, N-Myc, and L-Myc, which have been attracting growing interest for their function in stem cell regulation. In addition, evidence has established an emerging role of Myc in CSC regulation (50-53). Kruppel-like factor4, Klf4, is a protein member of the zinc finger-containing transcription factor family that regulates numerous cellular biological processes, such as cell differentiation, growth and proliferation, and apoptosis (54). In cancer, Klf4 has exhibited a role as a dual-function transcription factor,

Table IX. The ENCODE histone modifications 2015 top ten enriched transcription factors affected by DPTA NONOate.

Histone modification	p-Value	OR	Combined score	Targeted genes from input genes
H3K79me3_C2C12_mm9	0.008089	1.66	7.98	<i>GFM1; HSPA4L; RPL11; DDX41; GCSH; GRPEL2; HSF1; SNIP1; KNTC1; RPS2; CD3EAP; MRPS2; FTSJD2; KCTD3; WBP11; TNFRSF1A; FBXL12; MRPL51; UBE2S; MRTO4; TAF7; SNRPE; MAN1B1; PLP2; POLR3K</i>
H3K9me1_K562_hg19	0.026988	1.52	5.50	<i>EPHB6; ZNF782; GALNT6; LGI4; PLAC1L; GPR4; FTSJD2; SENP2; INHBC; FAM167B; DSCR4; ARL5B; PDP2; PTTG1; ICT1; GPD1; GRPEL2; CLDN23; HMG20B; SNRPE; PIK3C3; HMGXB3; IL17D</i>
H3K79me2_C2C12_mm9	0.046138	1.46	4.48	<i>GFM1; RPL11; MRPS2; DDX41; WBP11; FBXL12; RPS26; MRPL51; PDP2; PTTG1; ERCC3; UBE2S; ICT1; MRTO4; HSF1; SNIP1; KNTC1; SNRPE; MAN1B1; RPS2; PLP2; POLR3K</i>
H2AFZ_HepG2_hg19	0.046138	1.46	4.48	<i>G6PC; INO80D; SNAPIN; MYL6B; DDX41; FBXL12; RPS26; EFNBI; C12ORF45; MRPL51; KIAA1328; PDP2; UBE2S; MRTO4; POLR2D; C3ORF75; SURF6; TAF7; PIK3C3; HMGXB3; POLR3K; UGT2B7</i>
H3K36me3_thymus_mm9	0.083828	1.50	3.72	<i>SASH3; NDUFB10; RPL11; MRPS2; PDXP; THY1; FBXL12; RPS26; RPS27; UBE2S; GRAP2; ORMDL2; SNRPE; RPS2</i>
H3K27ac_MEL cell line_mm9	0.075402	1.39	3.59	<i>GFM1; SNAPIN; NDUFB10; HSPA4L; FBXO48; MRPS2; MYL6B; BAZ2B; ARHGAP15; RPS26; FYCO1; RPS27; PDP2; ICT1; POLR2D; HSF1; SNIP1; TAF7; KNTC1; SNRPE; PSTK</i>
H4K20me1_CD14-positive monocyte_hg19	0.075402	1.39	3.59	<i>ZNF782; SNAPIN; NFIX; NDUFB10; YIPF2; CD3EAP; MRPS2; PDXP; DDX41; OSD1; BRI3BP; KCTD9; EFNBI; ICT1; NRIP2; POLR2D; SNIP1; SURF6; HMG20B; RPS2; POLR3K</i>
H3K9ac_A549_hg19	0.075402	1.39	3.59	<i>ZNF782; PRRT1; MRPS2; MYL6B; DDX41; WBP11; SENP2; CELSR2; RCBTB1; ARL5B; KIAA1328; PER3; CCDC88C; HSF1; HMG20B; ZNF514; TAF7; POLR3K; ICOSLG; UGT2B7; ZNF354B</i>
H3K79me2_myocyte_mm9	0.075402	1.39	3.59	<i>GFM1; HSPA4L; RPL11; MRPS2; TNFRSF1A; FBXL12; RPS26; ARL5B; MRPL51; PDP2; ERCC3; UBE2S; MRTO4; HSF1; SNIP1; TAF7; KNTC1; SNRPE; PIK3C3; RPS2; POLR3K</i>
H3K36me3_Caco-2_hg19	0.163209	1.73	3.14	<i>RPS26; RPS27; UBE2S; TAF7; RPS2</i>

OR: Odds ratio.

whereby it acts as an onco-protein or a tumor suppressor protein depending on the type of cancer and stage of the disease (55). There is less clear information available on the function of Klf4 in CSCs regulation compared to the other three transcription factors. Since Klf4 showed a function as an onco-protein, it is believed to involve in the proliferation of CSCs (54).

According to the emerging role of epigenetics in the regulation of gene expression, several epigenetic modifiers have been extensively studied regarding their activity on CSC formation and maintenance (56). Histone and DNA modification control gene transcription by regulating the structure of chromatin and DNA, which allows or does not allow transcription factors to interact and promote the expression of their target gene (57, 58). miRNAs control gene expression by regulating the levels of its target miRNAs,

which regulate the self-renewal and differentiation of cancer stem cells (59). Herein, we found several potential histone modifications and miRNAs that could be the targets of NO. H3K36me3 was shown to have the highest degree of epigenetic modification hubs that were altered by NO exposure. H3K36me3 was also found to be associated with a poor prognosis in hepatocellular carcinoma (60) and correlated with cancer-related gene expression in cancer cell lines (61). In addition, H3K79Me3 is one of the histone modifications of interest due to controlling epithelial-to-mesenchymal (EMT) (62) and the stemness of certain cells (63). H3K9Me1 has exhibited capacity for liver cancer formation (64) and was found to be a prognosis marker in oral cancer and oropharyngeal squamous cell carcinoma (65).

Furthermore, the suggested miRNAs from bioinformatic analyses have been documented for their cancer-related

Table X. The TargetScan microRNA2017 top ten enriched transcription factors affected by DPTA NONOate.

miRNAs	p-Value	OR	Combined score	Targeted genes from input genes
hsa-miR-4749-5p	0.009069	1.88	8.84	<i>PRRT1; NFIX; AATK; PDXP; MYL6B; THY1; FAM167B; SFRP1; RCN1; GPD1; CLDN23; HSF1; SURF6; HMG20B; MAN1B1; ICOSLG; SDCCAG8</i>
hsa-miR-4706	0.009069	1.88	8.84	<i>PRRT1; NFIX; AATK; PDXP; MYL6B; THY1; FAM167B; SFRP1; RCN1; GPD1; CLDN23; HSF1; SURF6; HMG20B; MAN1B1; ICOSLG; SDCCAG8</i>
hsa-miR-615-3p	0.019615	2.24	8.83	<i>PRRT1; GALNT6; ZNF362; NFIX; POLR2D; HSF1; JOSD1; IL17D; CELSR2</i>
mmu-miR-3072	0.019402	1.64	6.46	<i>PRRT1; COLEC12; ZNF362; INO80D; LGI4; HSPA4L; CD3EAP; PDXP; MYL6B; JOSD1; CELSR2; RCBTB1; UBE2S; GRAP2; ORMDL2; KCNMB3; CASQ2; NUDT18; LONRF1; ICOSLG</i>
hsa-miR-28-3p	0.024211	1.60	5.96	<i>G6PC; INO80D; NFIX; FAM46A; FBXO48; JOSD1; SENP2; CELSR2; RCBTB1; DCBLD2; ARGFX; ARL5B; PLN; GRPEL2; POLR2D; ASB9; CASQ2; PRKCQ; LONRF1; RBMS2</i>
mmu-miR-187	0.055948	1.94	5.59	<i>KCTD9; INO80D; SNAPIN; PLN; PER3; FAM46A; LONRF1; RBAK</i>
mmu-miR-695	0.035791	1.67	5.55	<i>PRRT1; COLEC12; ZNF362; NFIX; LGI4; FAM46A; PDXP; JOSD1; CELSR2; TRHDE; EFNBI; ASB9; PRKCQ; PLP2; RBMS2</i>
hsa-miR-4781-5p	0.04838	1.77	5.35	<i>PRRT1; NFIX; LGI4; CEP164; POLR2D; HSF1; SURF6; THY1; CELSR2; INHBC; ICOSLG</i>
hsa-miR-572	0.074612	1.92	4.97	<i>RCN1; INO80D; NFIX; POLR2D; MRPS2; TAF7; BRI3BP</i>
mmu-miR-1947	0.081796	1.87	4.69	<i>KCTD9; EFNBI; INO80D; PER3; GRAP2; POLR2D; RBMS2</i>

OR: Odds ratio.

Table XI. The miTarBase2017 top ten enriched transcription factors affected by DPTA NONOate.

miRNAs	p-Value	OR	Combined score	Targeted genes from input genes
mmu-miR-292a-3p	0.002449	26.49	159.26	<i>PDP2; MYL6B</i>
mmu-miR-290a-3p	0.002978	24.08	140.07	<i>PDP2; MYL6B</i>
mmu-miR-467a-5p	0.002978	24.08	140.07	<i>PDP2; MYL6B</i>
hsa-miR-3648	0.009841	13.25	61.21	<i>NFIX; YIPF2</i>
mmu-miR-465c-3p	0.051675	18.92	56.06	<i>DCBLD2</i>
mmu-miR-465b-3p	0.051675	18.92	56.06	<i>DCBLD2</i>
mmu-miR-465a-3p	0.051675	18.92	56.06	<i>DCBLD2</i>
hsa-miR-6761-3p	0.051675	18.92	56.06	<i>NFIX</i>
mmu-miR-3086-5p	0.051675	18.92	56.06	<i>BRI3BP</i>
hsa-miR-4313	0.001655	7.91	50.64	<i>PER3; UBE2S; GRAP2; FBXO48</i>

OR: Odds ratio.

activities. miR-615-3p has been found to be target of Nanog/OCT4/TCF3 protein (66) and showed an association with cancer recurrence in hepatocellular carcinoma (67). miR-3648 has been identified to play a role in the regulation of both the proliferation (68, 69) and invasion (70) of certain cancers.

Transcription factors, histone modifications, and miRNAs have been shown to have roles in CSCs and CSC-related behaviors. Although certain molecular targets of NO for controlling tumor aggressiveness and the CSC phenotype have been elucidated, the analysis by whole mRNA expression with validated bioinformatics tools has not been

elucidated. Herein, we provide valuable data that demonstrate potential new targets of NO in regulating cancer progression that can benefit NO research and offer knowledge on cancer cell biology.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

Supervision: P.C.; Participated in research design: A.M. and P.C.; Conducted experiments: A.M.; Performed data analysis: A.M. and O.P.; Wrote or contributed to the writing of the manuscript: A.M. and P.C.

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Availability of Data and Material

The datasets used during the present study are available from the corresponding author upon reasonable request.

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