http://jbcp.shahed.ac.ir 10.22070/JBCP.2021.14817.1147

Open Access Research Article

Journal of Basic and Clinical Pathophysiology
Print ISSN: 2322-1895, **Online ISSN:** 2345-4334
Vol.9, No.2, December 2021

A Semiannual Publication of
Shahed University Press

Tobacco carcinogen-induced alterations in expression of cellular stress response genes in lung of Wistar rats and the effect of exercise training

Maryam Khalesi[1*], Shadmehr Mirdar[2], Ali Samadi[1]

1. Physical Education and Sport Sciences Department, Faculty of Humanities, Shahed University, Tehran, Iran
2. Faculty of Physical Education and Sport Sciences, University of Mazandaran, Babolsar, Iran

Abstract

Objective: Present study investigated the effect of tobacco-specific carcinogen, nicotine-derived nitrosamine ketone (NNK), on the expression of cellular stress response genes in lung tissue of Wistar rats. Moreover, the effect of exercise training on alterations of these genes in exposed rats was investigated.

Materials and Methods: A total of 30 healthy Wistar rats were obtained and divided into the following group: (1) Control (CON), (2) NNK exposure (NNK, once a week / 12.5 mg per kg body mass for 12 weeks), and NNK + swimming training (NNK+ST, received NNK exposure + 25-60 min of aerobic swimming training / 5 days per week for 12 weeks). The mRNA expression level of nuclear factor kappa-B (NF-κB), forkhead box protein O3 (FOXO3), and Sirtuin-1 (Sirt-1) were determined in lung tissue by the quantitative real-time PCR.

Results: The NNK exposure resulted in a significant reduction in mRNA expression of the NF-κB and FOXO3 (P=0.0001 and P=0.005, respectively), as well as a significant increment in mRNA expression of Sirt-1 (P=0.001) comparing to the CON group. Moreover, the ST in rats under NNK treatment led to a significant reduction in mRNA expression of NF-κB (P=0.0001), but it did not have any significant effect on mRNA expression of FOXO3 and Sirt-1 (P>0.05) comparing to the NNK group.

Conclusion: These findings indicate that NNK exposure can lead to adverse alterations in the expression of cellular stress response genes in the lung tissue of Wistar rats and exercise training may not have any favorable effect on the expression of these genes in rats under NNK treatment.

Keywords: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Cell stress, Pulmonary, Swimming training

1. Introduction

Nicotine-derived nitrosamine ketone (NNK) or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is the most abundant and powerful carcinogenic compound in tobacco smoke and exerts its pathological effects after metabolic activation by the cytochrome P450 enzymes (1,2). After bioactivation, NNK can lead to the formation of various deoxyribonucleic acid (DNA) adducts which if not repaired may result in incorrect nucleotide bindings, mutation, tumor growth, and progression (2,3). Moreover, NNK can bind to beta-adrenergic receptors (β-AdrR) and nicotinic acetylcholine receptors (nACHR) and activate signaling pathways that regulate cell proliferation, apoptosis and survival, as well as immunosuppressive pathways which can disrupt the normal cell cycle (2). Consequently, this can lead to the accumulation of "primer" cells that have mutations in their tumor suppressor genes and oncogenes required for cellular transformation (4). Due to the remarkable organospecificity of the NNK to lung, the incidence of lung diseases including chronic obstructive pulmonary disease and lung cancer is significantly higher among tobacco users, with 10% to 20% of smokers developing these diseases (5). However, the molecular and cellular

Revised: 25 Aug 2021, **Accepted:** 20 Oct 2021, **Available online:** 30 Dec 2021
Corresponding Author: Maryam Khalesi **E-mail:** m.khalesi@shahed.ac.ir

mechanisms underlying NNK pathogenesis in the lung have not yet been completely understood. Sirtuin-1 (Sirt-1) is a nicotinamide adenine dinucleotide–dependent deacylase which may function as an intracellular regulatory protein. Sirt-1 has complex functions and its effects on various processes including stress response, metabolism, and cancer development is still under investigation (5). It interacts with several other transcriptional factors including nuclear factor kappa-B (NF-κB) and forkhead box protein O3 (FOXO3) and inhibits or promotes their activity and plays an important role in the regulation of cell death/survival pathways (6). NF-κB is a transcription factor that is well-known for its role in the regulation of inflammatory response and promoting oncogenesis (4,7). Moreover, some studies highlighted the role of NF-κB in regulating cell apoptosis, especially in transformed cells. Research studies show that NF-κB may act as both tumor promoter and suppressor (8,9). FOXO3 is a member of forkhead box class O transcription factors which responds to several cellular stresses and mediates multiple physiological and pathological processes including DNA damage, apoptosis, and tumorigenesis. Furthermore, recent evidence suggests that FOXO3 may act as a tumor suppressor in cancer (10). Despite the critical role of these transcriptional factors and regulatory proteins in cell stress response and carcinogenesis, less is known about their response to NNK, which is the most potent tobacco smoke carcinogen.

Moreover, regular exercise training has been shown to have numerous health benefits and reduce cancer incidence, inhibit tumor growth and cancer progression through a direct effect on tumor-intrinsic factors, improving immune system function, and interplay with whole-body exercise effects (11,12). Although the exact mechanisms by which exercise training may inhibit tumor growth and cancer progression are not clear yet, we postulated that the preventative effects of exercise training may in part be mediated by regulating these transcriptional factors and regulatory proteins. Therefore, the present study aimed at investigating the mRNA expression of NF-κB, FOXO3, and Sirt-1 in lung tissue of NNK-exposed rats. Furthermore, the effect of swimming training on the mRNA expression level of these transcriptional factors and regulatory proteins in lung tissue of NNK-exposed rats was investigated.

2. Materials and Methods

In this experimental study, 30 male and female Wistar rats (6-8 weeks old, weighing 105±25 g) were purchased from the Laboratory of Animal Care of Pasteur Institute in Amol. All steps and procedures of the study were accordant with national guidelines for the use of laboratory animals and were approved by the Mazandaran University (code number: MUBABOL.HRI.REC.1395.109). Animals were housed in cages (four rats in each cage) made of transparent polyethylene in a 12:12 h light-dark cycle with ad libitum access to food and water (ambient temperature ~23 °C, humidity 45–60%). Then, they were divided into the following groups: (1) Control (C, n = 10); (2) NNK exposure (NNK, n = 10); (3) NNK exposure + swimming training (NNK+ST, n = 10). Calculation of sample size was done based on resource equation method in which: E = Total number of animals − Total number of groups, and any sample size which keeps E between 10 and 20 considered adequate (13) [in current study E = (10*3) – 3 = 27 which is more than 20. So, the sample size in the current study was more than necessary].

2.1. NNK exposure

Following the adaptation and familiarization, in the NNK and NNK+ST groups, the NNK was subcutaneously injected once a week for 12 weeks (12.5 mg/kg body mass) (14). Rats in the C group were injected with the same amount of distilled water. Rats in the NNK+ST group were treated with the NNK and exercised 5 days per week till the end of the study.

2.2. Exercise training protocol

Rats in the NNK+ST group were trained to swim in a rodent swimming pool (50 height × 50 widths × 100 length cm) for one week in the adaptation phase of the study (3 sessions, 10-20 min per session). Then the swimming training protocol was started from the third week along with the NNK exposure commencement. The aerobic swimming training was performed 25-60 min per session 5 days per week for 12 weeks. The workload increased gradually by increasing the duration (25 to 60 min) and water flow rate (4 to 10 lit per min) by the end of the seventh week. Then the workload was maintained till the end of the study (15) (Table 1).

Table 1. Swimming training protocol

Phase		Frequency (d/w^{-1})	Duration (minutes)	Intensity*
Adaptation	Week 1	-	-	-
Familiarization	Week 2	3	10 to 20	2
Main	Week 3 to 6	5	25 to 40	4 to 5
	Week 7 to 10	5	45 to 60	6 to 7
	Week 11 to 14	5	60	8 to 10

* Average flow rate (liter per minute)

2.3. Sample collection

Forty-eight hours after the last exercise training session rats were anesthetized with a combination of xylazine 10% and ketamine 2% and tissue samples were collected. The lower lobe of the right lung of all rats was isolated, quickly washed with phosphate-buffered saline, and immediately placed in a -70° C freezer until they were used for assessments.

2.4. RT-PCR analysis

RNA extraction was performed manually by QIAzol® Lysis Reagent (Qiagen, Germany) and chloroform (Qiagen, Germany). Then, the RNA concentration obtained from 50 mg of lung tissue in each homogenized sample was assessed (Eppendorf, Germany). The ratio of 260 to 280 (between 1.8 and 2) was defined as the optimal concentration of RNA. In the second step, RNA samples were incubated to remove genomic DNA under specific conditions. The cDNA was synthesized using the QuantiTect Reverse Transcription Kit (Qiagen, Germany) according to the manufacturer's instructions. After cDNA synthesis, a polymerase chain reaction (PCR) was performed. The mRNA levels of NF-κB, FOXO3, and Sirt1 in lung homogenates were assessed by quantitative Real time-PCR (using PriMix SYBER Green II) (Applied Biosystems Step One, USA). In this method, the reaction mixture was performed in a final volume of 20 Landa and each reaction was duplicated. The Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal reference control. The mRNA expression levels were calculated using the comparative CT method ($2^{-\Delta\Delta CT}$) (16). All data are presented as fold change relative to control. The primer sequences of the genes are presented in Table 2.

Table 2. The primer sequences of the genes

Gene	Primer sequences
NF-κB	FOR:5´- CATACGCTGACCCTAGCCTG -3´ REV:5´- TTTCTTCAATCCGGTGGCGA -3´
FOXO3	FOR:5´- GCCTCATCTCAAAGCTGGGT-3´ REV:5´- TGCTCTGGAGTAGGGATGCT -3´
Sirt-1	FOR:5´- TGGTTTACAACGTCTGTGCCT -3´ REV:5´- GCTGCTTGCTGTCCATACCT -3´
GAPDH	FOR:5´- AAGTTCAACGGCACAGTCAAGG -3´ REV:5´- CATACTCAGCACCAGCATCACC -3´

2.5. Statistical analysis

The Kolmogorov–Smirnov was used to test the normality of data. One-way analysis of variance (ANOVA) was performed to identify significant differences ($P<0.05$) among groups, which followed by Dunnett's T3 post hoc test when a significant difference was observed. Statistical analysis was conducted using IBM SPSS Statistics 20 (IBM, New York, NY, USA).

3. Results

Table 3 represents the body mass changes of the groups throughout the study.

Table 3. Body mass (mean±SD) of study groups (gram)

Phase	Time	Group		
		Con	NNK	NNK+ST
Adaptation	Week 1	103.6±10.2	106.7±20.1	111.6±35.9
Familiarization	Week 2	127.4±12	138.1±19.9	124.7±35
Main	Week 3	146.6±8.7	162.5±21.5	155.7±26.6
	Week 4	163.4±9.1	186.1±27.2	173±12.9
	Week 5	175.6±9.8	209.5±34.1	192.2±13
	Week 6	186.2±8.6	223.6±39	208.4±16.3
	Week 7	197±8.3	240±42.5	225±24.6
	Week 8	205.6±7.7	241.6±42.4	226.7±21.9
	Week 9	206±7.5	258±50.6	236.4±31.5
	Week 10	210.4±11.2	264.4±54.5	246±38.4
	Week 11	218.2±8.8	272.6±56.6	261.7±45.4
	Week 12	221.6±8.9	274.7±61.6	282.2±63.4
	Week 13	226.5±6.4	288.3±64.1	277.5±59
	Week 14	259.5±9.3	285.6±70.4	274.3±61.8

CON= Control; NNK= NNK exposure; NNK+ST= NNK exposure + swimming training

Effect of NNK treatment and exercise training on NF-κB, FOXO3a, and Sirt-1

The results showed that NNK treatment led to a significant decrease in mRNA expression of FOXO3 (P=0.005) (Figure 1A) and NF-κB (P<0.0001) (Figure 1B), while it resulted in a significant increase in expression of Sirt-1 (P = 0.001) (Figure 1C). Moreover, the result revealed that cotreatment with NNK+SE resulted in a significant increase in mRNA expression of Sirt-1 (P<0.0001) (Figure 1C) and a significant decrease in NF-κB (P<0.0001) (Figure 1B) and FOXO3 (P=0.006) (Figure 1A) expression compared to the CON group. Finally, comparing the NNK and the NNK+SE group showed that exercise training in rats under NNK treatment led to a significant decrease in the expression of NF-κB (P<0.0001) (Figure 1B), but it did not have any significant effect on mRNA expression of the FOXO3 (P=0.887) and Sirt-1 (P=0.1) (Figure 1A and 1C, respectively).

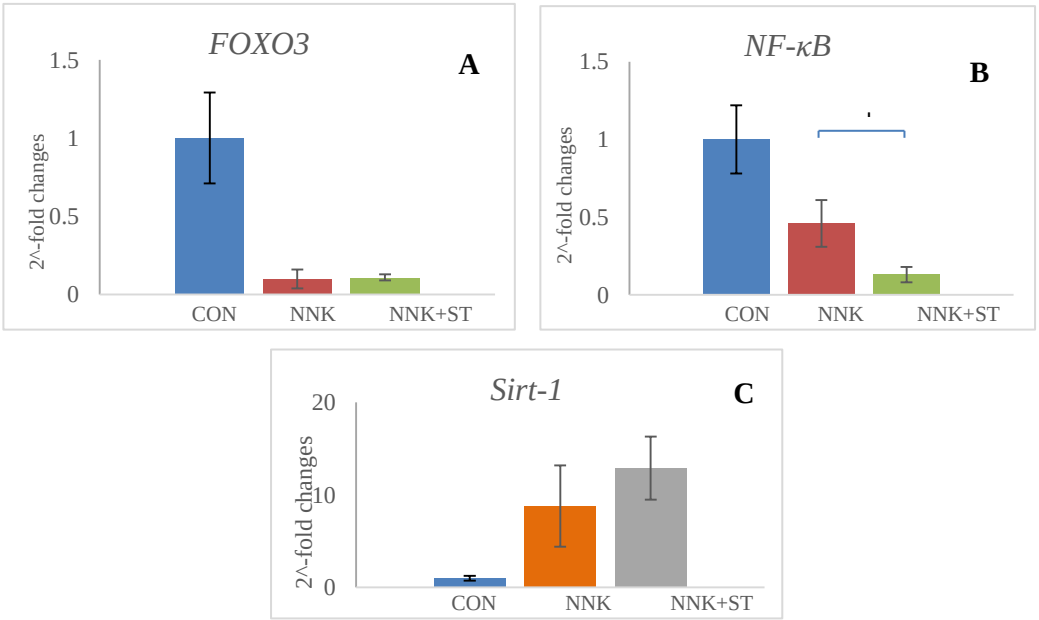

Figure 1. 2^-fold changes in relative expression of FOXO3 (A), NF-κB, (B), and Sirt-1 (C) in study groups. CON= Control; NNK= NNK exposure; NNK+ST= NNK exposure + swimming training.
* Significant change in expression as compared to the CON group p<0.05. ǂ Significant change in expression as compared to the NNK group p<0.05.

4. Discussion

NNK is one of the most potent carcinogens in tobacco products that affect various cellular processes including cell survival/apoptosis and causes cellular injury in various tissues including the lung tissue. The results of the present study showed that NNK exposure for 12 weeks led to a significant reduction in mRNA expression of the NF-κB in lung tissue of rats. NF-κB is a transcription factor supporting host responses to cellular stress and immune responses to pathogens and other challenges, as well as it plays opposing functions in inflammation and cancer (17). Some studies have shown that NF-κB has an oncogenic role in cancer development through induction of expression of genes involved in the blockade of apoptosis, promotion of proliferation, and angiogenesis which all contribute to the malignant transformation of cells (18). However, other studies reported that NF-κB is involved in tumor suppression (19). These opposing roles might be depended on cell type. Although the tumorigenesis did not assess in the current study, previous studies have proved the role of NNK in DNA damage and tumor development with the dose and time course applied in the present study (14), so the decreased NF-κB expression observed in the current study after NNK exposure supports the anti-tumoral role of NF-κB in lung cancer development. Moreover, considering the crucial role of the NF-κB in inflammation and immune responses the decreased expression of the NF-κB due to NNK treatment means that along with DNA damage NNK probably leads to suppression of immune responses necessary for removal of damaged cells, which consequently results in accumulation of transformed cells and tumor progression in lung tissue. However, further research is needed to provide more evidence before drawing any conclusion. Our results also showed that NNK exposure reduced mRNA expression of FOXO3 in lung tissue of Wistar rats. Although some previous studies reported a tumorigenic role for FOXO3 (20,21), recent studies classified it as a tumor suppressor and it has been shown that deregulation of FOXO3 is associated with cancer development (22,23). Previous studies have reported decreased FOXO3 in various types of cancers including breast (24), prostate (25), and bladder (26). Moreover, a more recent study also reported that FOXO3 expression was suppressed in lung cancer tissues and cells (27). As NNK is a potent carcinogen and previous studies proved its tumorigenic potency with the same dose used in the present study (14), decreased expression of FOXO3 after NNK treatment that observed in the present study provides further evidence that FOXO3 plays an anti-tumoral role in lung cells. It also suggests that tumorigenic effects of NNK in lung cells and tissue may in part be due to the deregulation of FOXO3 expression. Additionally, our result revealed that exposure to NNK for 12 weeks led to a significant increase in mRNA expression of Sirt-1 in lung tissue of Wistar rats. In line with our finding Jianyi Lu et al., (2015) showed that Benzo[a]pyrene (B[a]P) exposure, a carcinogen in cigarette smoke, led to overexpression of Sirt-1 in the lung of mice (28). In normal cells, Sirt-1 plays major roles in processes such as cell cycle, metabolism, DNA repair, aging, and cell survival (29). However, Sirt-1 can also function as an oncogene through the regulation of some tumor suppressors, including p53 and FoxOs, and NF-κB (30). In fact, a recent meta-analysis concluded that Sirt-1 overexpression was significantly correlated with overall survival in different cancers including lung and liver cancers and its overexpression indicates poor prognosis in these patients (31). Moreover, it has been shown that silencing the Sirt-1 gene can inhibit the migration or invasion of cancer cells (32-34), which suggests its role in tumor progression and metastasis. Considering the significant increase in Sirt-1 expression in the present study due to NNK exposure it seems that our findings are consistent with the oncogenic role of Sirt-1 and NNK exposure probably has led to tumor induction in lung tissue of the rats. Finally, findings showed that swimming exercise in Wistar rats under NNK treatment led to a significant reduction in NF-κB expression, a non-significant increase in FOXO3 and Sirt-1 expression. To the best of our knowledge, this is the first study that investigates the effect of exercise training on the expression of these genes in subjects under NNK treatment. We postulated that swimming training in rats under NNK treatment may reduce pathogenic effects of NNK in lung tissue by improving cellular stress response or other mechanisms such as improving immune system function and enhancing antioxidant capacity, which all may increase the resistance of lung tissue against tumorigenic effects of NNK. Exercise-induced changes in expression of FOXO3 suggest a probable positive effect of exercise training in subjects under NNK treatment, however, the amount of changes was not statistically significant. On the other, considering the tumor-suppressive effects of NF-κB, significant decrease of NF-κB, and increased Sirt-1 expression following exercise in rats under NNK treatment indicates that exercise has exacerbated the negative effects of the NNK and may potentiate its pathogenic effects. In line with this finding, Ardies et al., (1996) showed that running exercise increased the expression of NNK-activating enzymes in lung tissue of rats (35). Possible mechanisms for the observed negative effects may be the increased NNK delivery to the lung tissue due to increased pulmonary perfusion during exercise, immunosuppressive effect of exercise because of high volume and frequency of exercise (5 days per week, up to 60 min per session) and imposing oxidative and physiological stress beyond adaptive capacity of the cells which all may result in increased vulnerability of lung to pathogenic effects of the NNK.

Conclusion

In summary, findings showed deregulation of stress response genes, namely Sirt-1, FOXO3, and NF-κB in lung tissue of rats exposed to NNK which could facilitate tumor initiation and progression in lung tissue. Moreover, findings indicated that exercise training in rats exposed to NNK had no beneficial effect on the expression of assessed stress response genes and even provided evidence of the plausible adverse effect of exercise, at least with duration, frequency, and intensity used in the present study, which might exacerbate the tumorigenic effects of the NNK in lung tissue.

Acknowledgements

We appreciate Dr. Mazaheri for her technical help in gene expression analysis.

Funding and support

The study was supported by Mazandaran University

Conflict of Interest

The authors declare no conflict of interest.

References

1. Vancou Neves Cruz J, Santana de Oliveira M, Gomes Silva S, Pedro da Silva Souza Filho A, Santiago Pereira D, Lima e Lima AH, et al. Insight into the interaction mechanism of nicotine, NNK, and NNN with cytochrome P450 2A13 based on molecular dynamics simulation. Journal of Chemical Information and Modeling 2019; 60(2):766-76.
2. Xue J, Yang S, Seng S. Mechanisms of cancer induction by tobacco-specific NNK and NNN. Cancers 2014; 6(2):1138-56.
3. Li L, Megaraj V, Wei Y, Ding X. Identification of cytochrome P450 enzymes critical for lung tumorigenesis by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK): insights from a novel Cyp2abfgs-null mouse. Carcinogenesis 2014; 35(11):2584-91.
4. Cai Z, Tchou-Wong KM, Rom WN. NF-kappaB in lung tumorigenesis. Cancers 2011; 3(4):4258-68.
5. Beane J, Cheng L, Soldi R, Zhang X, Liu G, Anderlind C, et al. SIRT1 pathway dysregulation in the smoke-exposed airway epithelium and lung tumor tissue. Cancer Research 2012; 72(22):5702-11.
6. Ren Z, He H, Zuo Z, Xu Z, Wei Z, Deng J. The role of different SIRT1-mediated signaling pathways in toxic injury. Cellular & Molecular Biology Letters 2019; 24(1):1-0.
7. Ren D, Yang Q, Dai Y, Guo W, Du H, Song L, et al. Oncogenic miR-210-3p promotes prostate cancer cell EMT and bone metastasis via NF-κB signaling pathway. Molecular Cancer 2017; 16(1):1-6.
8. Tabruyn SP, Griffioen AW. NF-κB: a new player in angiostatic therapy. Angiogenesis 2008; 11(1):101-6.
9. Baud V, Karin M. Is NF-κB a good target for cancer therapy? Hopes and pitfalls. Nature Reviews Drug Discovery 2009; 8(1):33-40.
10. Yang W, Du WW, Li X, Yee AJ, Yang BB. Foxo3 activity promoted by non-coding effects of circular RNA and Foxo3 pseudogene in the inhibition of tumor growth and angiogenesis. Oncogene 2016; 35(30):3919-31.
11. Hojman P, Gehl J, Christensen JF, Pedersen BK. Molecular mechanisms linking exercise to cancer prevention and treatment. Cell Metabolism 2018; 27(1):10-21.
12. Song M, Chan AT. The potential role of exercise and nutrition in harnessing the immune system to improve colorectal cancer survival. Gastroenterology 2018; 155(3):596-600.
13. Charan J, Kantharia ND. How to calculate sample size in animal studies?. Journal of Pharmacology & Pharmacotherapeutics 2013; 4(4):303-306.
14. Lao Y, Yu N, Kassie F, Villalta PW, Hecht SS. Formation and accumulation of pyridyloxobutyl DNA adducts in F344 rats chronically treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of its metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Chemical Research in Toxicology 2007; 20(2):235-45.
15. Khalesi M, Mirdar S, Samadi A. Effect of a period of swimming exercise on Sirt1 and FoxO3a genes expression in lung tissue of Wistar rats. Journal of Sabzevar University of Medical Sciences 2018; 25(2):251-8.
16. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. Nature protocols. 2008;3(6):1101-8.
17. Antonangeli F, Natalini A, Garassino MC, Sica A, Santoni A, Di Rosa F. Regulation of

PD-L1 Expression by NF-κB in Cancer. Frontier in Immunology 2020:584626.
18. Park MH, Hong JT. Roles of NF-κB in cancer and inflammatory diseases and their therapeutic approaches. Cells 2016; 5(2):15.
19. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-κB as the matchmaker. Nature Immunology 2011; 12(8):715.
20. Lu M, Xiang J, Xu F, Wang Y, Yin Y, Chen D. The expression and significance of pThr32-FOXO3a in human ovarian cancer. Medical Oncology 2012; 29(2):1258-64.
21. Kim H, Kim CH, Kim JH, Lee JM, Jeon JH, Lee JY. Solidago virga-aurea extract induced apoptosis of breast cancer cells through FOXO3 mediated bim expression. Cancer Research 2016: 76(14 Supplement):3520-3520.
22. Liu Y, Ao X, Ding W, Ponnusamy M, Wu W, Hao X, et al. Critical role of FOXO3a in carcinogenesis. Molecular Cancer 2018; 17(1):1-2.
23. Zhang Y, Zhao H, Zhang L. Identification of the tumor-suppressive function of circular RNA FOXO3 in non-small cell lung cancer through sponging miR 155. Molecular Medicine Reports 2018; 17(6):7692-700.
24. Liu H, Song Y, Qiu H, Liu Y, Luo K, Yi Y, et al. Downregulation of FOXO3a by DNMT1 promotes breast cancer stem cell properties and tumorigenesis. Cell Death & Differentiation 2020; 27(3):966-83.
25. Dubrovska A, Kim S, Salamone RJ, Walker JR, Maira SM, García-Echeverría C, et al. The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proceedings of the National Academy of Sciences of the U S A 2009; 106(1):268-73.
26. Yu C, Zhang Z, Liao W, Zhao X, Liu L, Wu Y, et al. The tumor-suppressor gene Nkx2. 8 suppresses bladder cancer proliferation through upregulation of FOXO3a and inhibition of the MEK/ERK signaling pathway. Carcinogenesis 2012;33(3):678-86.
27. Cao Y, Li P, Wang H, Li L, Li Q. SIRT3 promotion reduces resistance to cisplatin in lung cancer by modulating the FOXO3/CDT1 axis. Cancer Medicine 2021; 10(4):1394-404.
28. Lu J, Zhang M, Huang Z, Sun S, Zhang Y, Zhang L, et al. SIRT1 in B [a] P-induced lung tumorigenesis. Oncotarget 2015; 6(29):27113.
29. Alves-Fernandes DK, Jasiulionis MG. The role of SIRT1 on DNA damage response and epigenetic alterations in cancer. International Journal of Molecular Science 2019; 20(13):3153.
30. Guan Y, Rao Z, Chen C. miR-30a suppresses lung cancer progression by targeting SIRT1. Oncotarget 2018; 9(4):4924.
31. Wang C, Yang W, Dong F, Guo Y, Tan J, Ruan S, et al. The prognostic role of Sirt1 expression in solid malignancies: a meta-analysis. Oncotarget 2017; 8(39):66343.
32. Han L, Liang XH, Chen LX, Bao SM, Yan ZQ. SIRT1 is highly expressed in brain metastasis tissues of non-small cell lung cancer (NSCLC) and in positive regulation of NSCLC cell migration. International Journal of Clinical and Experimental Pathology 2013; 6(11):2357.
33. Nakane K, Fujita Y, Terazawa R, Atsumi Y, Kato T, Nozawa Y, et al. Inhibition of cortactin and SIRT1 expression attenuates migration and invasion of prostate cancer DU145 cells. International Journal of Urology 2012; 19(1):71-9.
34. Byles V, Zhu L, Lovaas JD, Chmilewski LK, Wang J, Faller DV, et al. SIRT1 induces EMT by cooperating with EMT transcription factors and enhances prostate cancer cell migration and metastasis. Oncogene 2012; 31(43):4619-29.
35. Ardies CM, Smith TJ, Kim S, Yang CS. Induction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) activation in rat lung microsomes by chronic ethanol consumption and repeated running exercise. Cancer letters 1996;103(2):209-18.