Antitumor efficacy of atorvastatin in lung cancer mice model

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ABSTRACT

One of the most prevalent cancers in the world is lung cancer. Various factors, multiple inherited, and acquired mechanisms contribute to lung carcinogenesis. The cholesterol-lowering medication atorvastatin (ATOR) has been demonstrated to have additional intriguing biological uses, such as anticancer effects. This study aimed to evaluate the effects of ATOR treatment against lung cancer in mice model. Forty-five male CD-1 mice were divided into 3 groups (n = 15) as the follows: Group 1 (G1) was used as a normal control. Gps 2 and 3 were administered with urethane (Ure) (1mg/g) and butylated hydroxytoluene (BHT) (200 mg/kg) for lung cancer initiation and promotion, respectively. G2 was left as a cancer-bearing group. G3 was post-treated with 10 mg/kg ATOR. The results showed that the treatment of Ure/BHT-administered mice with ATOR led to a significant decreased in the tumour incidences, multiplicities, and sizes as compared with each treatment alone. Further, the treatment with ATOR has significantly induced apoptosis in lung tumour cells without adverse side effects.

Keywords: Atorvastatin, Urethane, Butylated hydroxytoluene, Lung cancer, Apoptosis.

1. Introduction

The second most common malignancy in the world, lung cancer is thought to be the primary cause of cancer-related mortality. Although smoking is the primary cause, other variables also play a role in the development of lung cancer. Furthermore, both hereditary and acquired causes of lung cancer susceptibility have been postulated (Shehata et al., 2023). Two histologic classes of lung cancer are well known: small-cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC) (Jones and Baldwin 2018). Depending on the type and stage of the lung cancer, there are several treatment options for it, including surgery, radiation, chemotherapy, and targeted therapy (Lemjabbar-Alaoui et al., 2015). The routes and features of various tumor entities will determine whether a new revolution in neoplastic cancer or targeted medications is created (Abbas and Rehman, 2018). When anti-cancer medications are combined, their combined effect is greater than when they are taken alone because they work together to target important pathways. These fight most malignancies by simultaneously targeting several cell-survival pathways and delaying the development of resistance (Bayat Mokhtari et al., 2017) .

It is possible that statins could be utilized to treat cancer because they appear to have an anti-inflammatory effect and have been shown to have anticancer properties in both experimental and epidemiological studies (Zhang et al., 2020; Grabarek et al., 2021). ATOR, a statin medication, is likely the anti-

cholesterol medication that is prescribed the most globally (Adams et al., 2015). Remarkably, hydroxy-3-methylglutaryl-coenzyme reductase is blocked by statins. This has pleiotropic effects on the several genes implicated in the etiology of lung cancer (Marcianò et al., 2022). Clinical applications of ATOR in SCLC patients with dyslipidemia have been reported (Kong et al., 2023). A growing body of preclinical and epidemiological research has demonstrated that dyslipidemia is a significant risk factor for the development of cancer. Statins have also been demonstrated to increase the sensitivity of the body to anticancer medications and may have the ability to prevent or treat cancer (Liu et al., 2023). This study addressed the molecular and biochemical mechanisms of the antitumor efficacy of the treatment with ATOR in the lung cancer mice model.

2. Materials and Methods

Chemicals and drugs

The atorvastatin (Lipitor 80 mg pills) was supplied by Pharma Check Company (Cairo, Egypt). We bought the PCNA rabbit polyclonal antibody from Novus Biologicals in the United States.

Mice

Male CD-1 mice (25 ± 5 g) were purchased from the holding company for biological products and vaccines (VACSERA), Helwan-Giza, Egypt. Mice were housed at room temperature (24 ± 2 °C) for a two-week acclimation period (5 per cage), with 12 hours dark 12 hours light cycle during the Spring/Summer seasons. Diet and water were available *ad libitum*. The animals were maintained in the animal facility of the Zoology Department at Tanta University under the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals (8th Edition 2011). The Institutional Animal Care and Use Committee (IACUC) of the Faculty of Science, Tanta University, approved the experimental design.

Experimental protocol

Forty-five male Swiss albino mice were divided into 3 groups (n = 15): Group 1 was intraperitoneally (i.p.) administered with saline (0.9 %) as normal controls. Groups 2 and 3 were i.p. administered with a single dose of Ure (1 mg/g), and 7 days later, they received a weekly single injection of BHT for 6 weeks; the 1st dose was 150 mg/kg, while the five later doses were 200 mg/kg (Salim et al., 2022). Following the last BHT injection, G2 was kept as a carcinogen-only- -administered control group. G3 was i.p. administered with ATOR (10 mg/ kg) (Fırıncı et al., 2014). Weight increase, absolute and relative lung weights, and beginning and end body weights were all measured. All animals were put to sleep with diethyl ether after 13 weeks. Samples of serum and blood were taken for biochemical and hematological examinations. The lungs were evaluated for tumors and histological studies after being necropsied, cleaned with isotonic saline solution, and injected with buffered formalin from the trachea opening for optimal fixing.

Histopathological investigations

Formalin (10%) with phosphate buffer was used to fix the lung tissues. Hematoxylin and eosin were used as a routine stain after the fixed tissues were embedded in paraffin. A stage and ocular micrometers were used to quantify the gross tumor incidences and multiplicities; the ocular micrometer was calibrated using the standard stage micrometer (Salim et al., 2023).

Flow cytometry analysis

Aliquots of homogenate cells were scraped from the lung of mice from different groups, suspended in cold phosphate-buffered solution (PBS) for flow cytometer, using fluorescence-activated cell sorting (FACS) caliber flow cytometer, (Becton Dickinson, Sunnyvale, CA, USA). The histogram derived from flow cytometry was obtained with a computer program for Dean and Jett mathematical analysis (Dean and Jett, 1974).

Statistical analysis

The statistically significant differences between samples were evaluated using the student's *t-test*. $P < 0.05$ were considered statistically significant. GraphPad Prism (version 8.3.0) instant software (San Diego, CA, USA) was used for statistics.

3. Results

Effect of the treatment with ATOR on the body and lung weight in mice

Table 1 showed the initial or final body weights as well as the relative and absolute lung weights. When comparing the end body weights of the groups to either the Ure + BHT-administered group (G2) or the normal control body weights (G1), there were no discernible differences. When compared to tumor-bearing groups alone, the ATOR-treated group experienced the greatest weight gain. Conversely, there was no significant difference in the absolute lung weights across all groups; however, G3 (Ure + BHT + ATOR) had considerably higher relative lung weights than G2 ($P < 0.05$).

Treatment with ATOR improved the lung nodules induced by Ure/BHT

Visible hyperplastic and neoplastic lesions were identified as lung nodules that developed on the lung exterior surfaces of mice given Ure/BHT (Fig. 1 A, B). The lung nodules in the normal control group were absent. Nevertheless, lung nodules in G2 injected with Ure/BHT alone were seen in the lungs of 12 out of 12 mice (100% occurrence). After receiving ATOR treatment, lung nodules harboring mice were found in 69.2% of the mice (9 out of 13 mice). In G2, the multiplicities of these lung nodules were 6.7 ± 1.3. There is a noticeable inhibitory impact from ATOR treatment (Fig. 1, D).

Tumor incidences (%), multiplicities, and average tumor sizes after ATOR treatment

in all Ure/BHT administered groups, the ocular micrometer was used to determine the tumor incidences and multiplicities (total numbers of adenomas and carcinomas after histopathological diagnosis) per 1 cm^2 of histological sections (Fig. 2) as well as the average square areas (sizes) of lung lesions (mm^2 per 1 cm^2) microscopically (Table 2). The lung sections of G1's negative control showed no histological abnormalities. After histopathological examination, G2 given Ure/BHT only shown a 100% incidence of adenomas and carcinomas; the total number of histological neoplasia per 1 cm2 of lung tissue was 9.1 ± 2.13. After receiving ATOR treatment, the incidence of lung tumors in G3

patients decreased significantly to 76.9%, with average numbers/1cm2 of 3.6 ± 1.7 (Table 2).

Treatment with ATOR increased the percentages of apoptotic lung cancer cells

The percentages of early and late apoptotic cells in the carcinogen control group (G2) were found to be considerably higher than the normal control values ($P < 0.05$) according to flow cytometry analysis. When compared to G2, treatment with ATOR significantly increased the number of early and late apoptotic cells ($P < 0.05$) (Fig. 3).
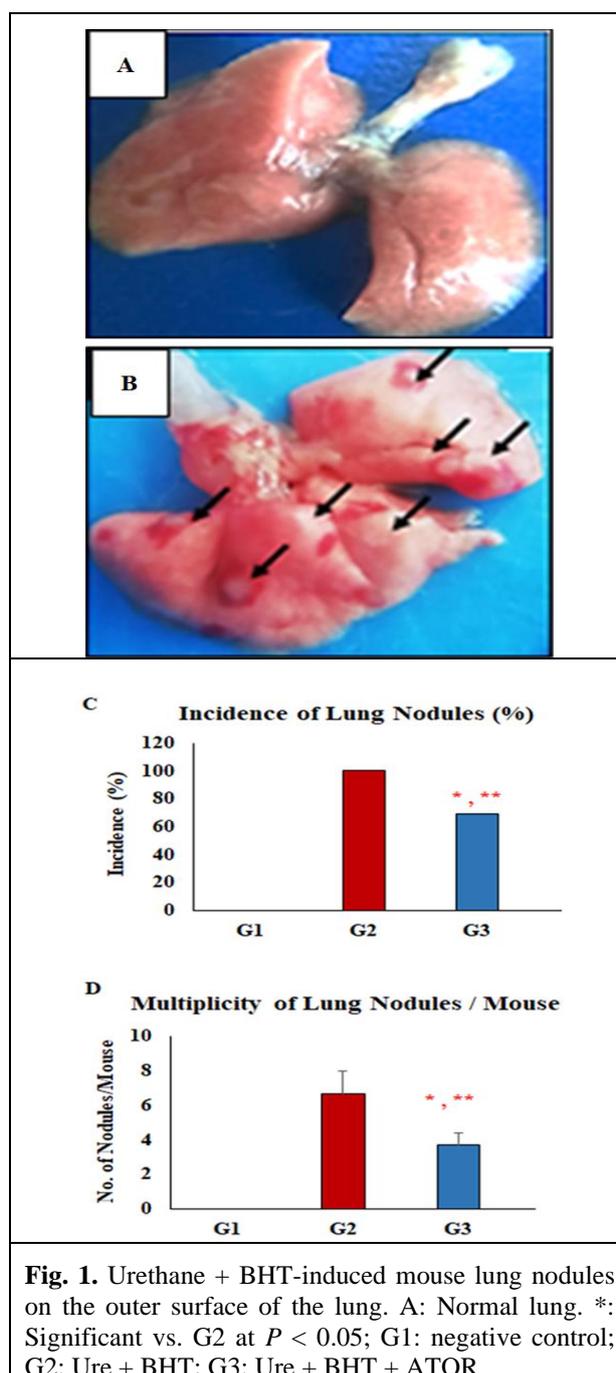

Fig. 1. Urethane + BHT-induced mouse lung nodules on the outer surface of the lung. A: Normal lung. *: Significant vs. G2 at *P* < 0.05; G1: negative control; G2: Ure + BHT; G3: Ure + BHT + ATOR

Table 1. Means of initial, and final body weights, weight gain, absolute and relative lung weights

Groups	G1 (-Ve control)	G2 (Ure/BHT)	G3(Ure/BHT/ATOR)
Mice's initial No.	15	25	25
Mice's final No.	13	20	21
Initial b. wt (g)	26.6 ± 3.4	28 ± 2.8	25.9 ± 2.7
Final b. wt (g)	36.8 ± 2.7	39.1 ± 4.4	36.3 ± 7.7
Weight gain (g)	10.2 ± 2.6	11.1 ± 5.4	10.4 ± 3.4
Lung wt (g)	0.31 ± 0.06[1] (0.83)[2]	0.33 ± 0.04 (0.84)	0.35 ± 0.6 (0.97) *

[1]: values of absolute organ wt. (means ± S. D.); [2]: values in parenthesis are relative organ wt: ratio of organ wt/ body wt. (%); *: significant vs. G2 at $P < 0.05$; **: significant vs. G3 at $P < 0.05$.

Table 2. Lung tumor incidences (%), multiplicities, and average of tumor areas.

Groups	Tumor Incidence [a] (%)	Tumor Multiplicity [b] (Tumor no./ 1 cm^2)	Tumor sizes (Tumor no./ 1 cm^2)
G1	0	0	0
G2	12/12 (100%)	9.1 ± 2.1	0.58 ± 0.07
G3	10/13 (76.9%)*	3.6 ± 1.7*	0.32 ± 0.03*

a: incidence of tumors (%) histopathological diagnosed as adenoma or adenocarcinoma; **b:** multiplicity of adenomas or adenocarcinomas counted per 1cm^2 of lung tissue; each reading represents means ± S.D. of triplicate observations; ***:** significant vs. G2 at $P < 0.05$; ****:** significant vs. G3 at $P < 0.05$.
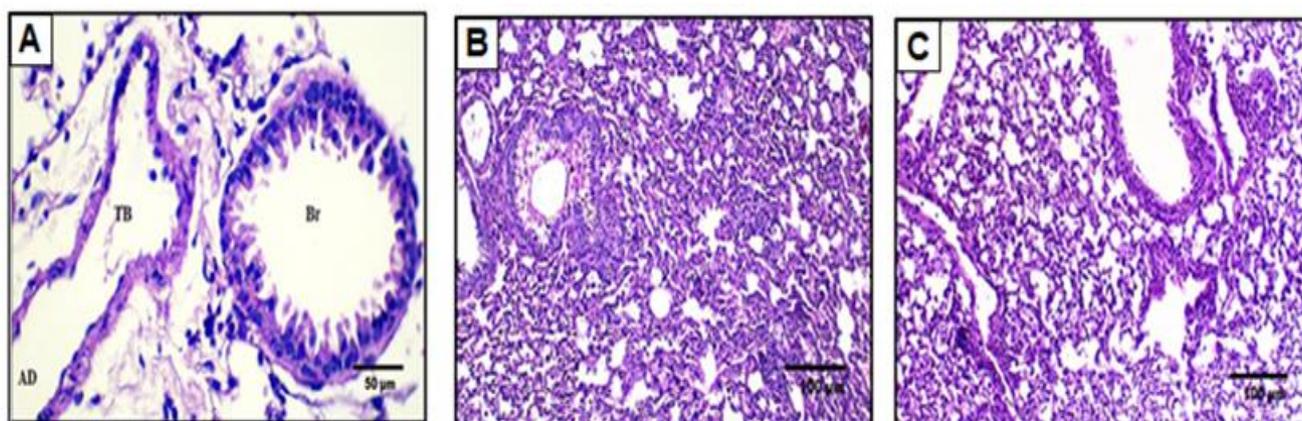

Fig. 2. Photomicrographs showing the histology of the lung epithelium and tumors in the five groups of mice. **(A) G1**: negative control; **(B) G2**: Ure + BHT; **(C) G3**: Ure + BHT + ATOR.

A

Early and Late Apoptotic cells (%) in lung mouse
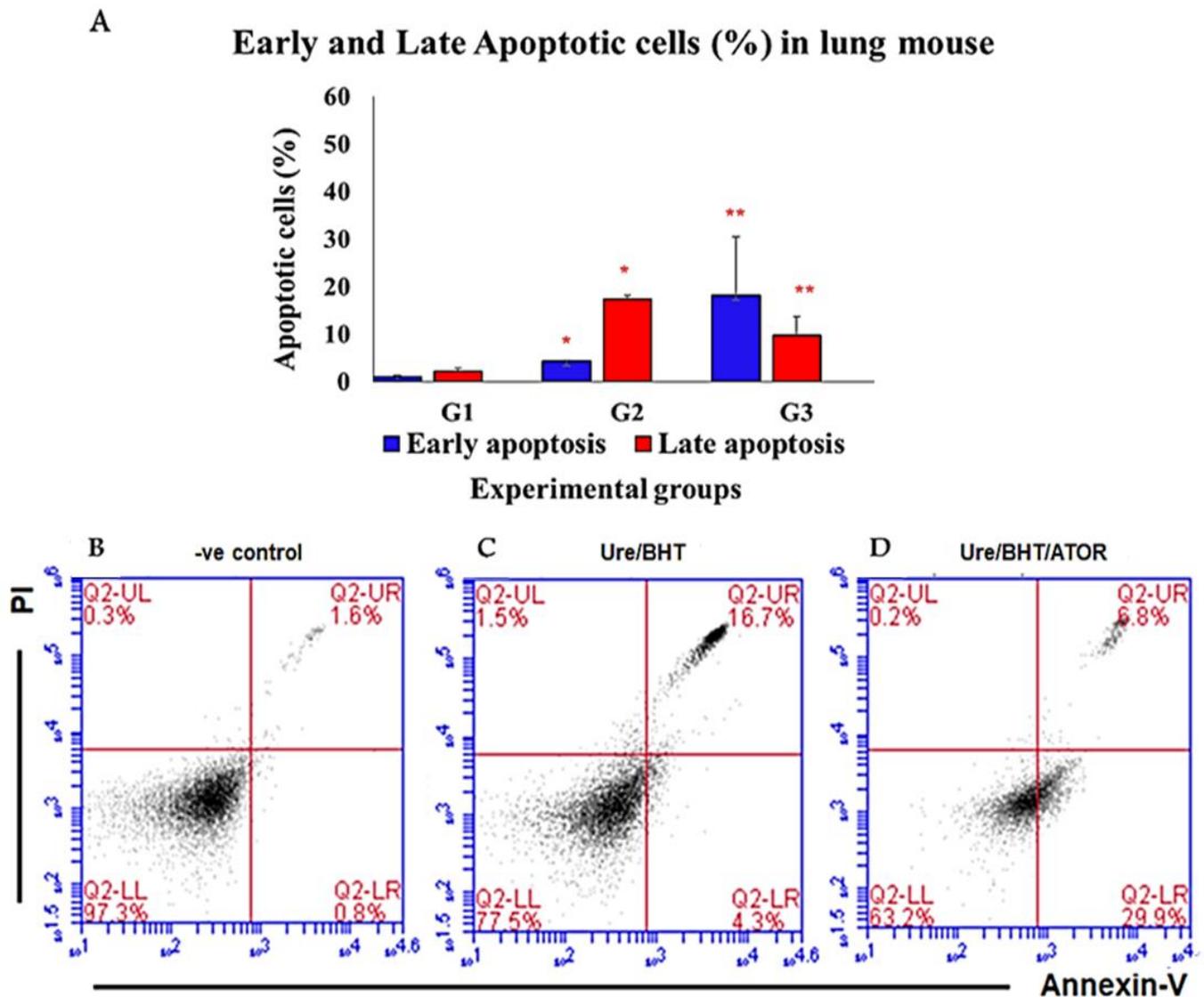

B -ve control

C Ure/BHT

D Ure/BHT/ATOR

Fig. 3. Flowcytometric analysis data for annexin V staining (n = 6). Values are shown as percentage means ± S.D; **G1**: negative control; **G2**: Ure + BHT; **G3**: Ure + BHT + ATOR; *****: Significant vs. G1 at $P < 0.05$; ******: Significant vs. G2 at $P < 0.05$; *******: significant vs. G3 at $P < 0.05$.

4. Discussion

It has long been believed that cancer is a hereditary disease that progresses due to a variety of mutations. Most well-studied tumor suppressor genes or oncogenes maintain the altered metabolic state that cancer has. (Gyamfi et al., 2022). Tumors arising in the lung parenchyma or within the bronchi are referred to as lung cancer, or bronchogenic carcinoma. It is among the main reasons why people die from cancer. The two main risk factors for lung cancer are smoking and exposure to various chemicals (Klebe et al., 2019). The risk is increased by lung conditions such idiopathic pulmonary fibrosis. The preferred treatment of lung cancer is surgery followed by adjuvant chemotherapy (Siddiqui et al., 2023).

In addition to lowering blood cholesterol levels and lowering the risk of cardiovascular disease, atorvastatin (ATOR) may be crucial in the early detection and management of lung cancer. Statins feature several anticancer characteristics, such as the capacity to lessen invasion, lower angiogenesis and cell proliferation, and jointly control the progression of lung cancer (Amin et al., 2021). In a lung cancer mouse model

initiated with urea-BHT, the purpose of this work was to investigate the possibility of using atorvastatin, an anti-cholesterol medication, as an anticancer therapy. The underlying molecular and metabolic mechanisms of their anticancer activity against the etiology of lung cancers were also emphasized in the study.

The current findings demonstrated that giving ATOR to mice did not significantly affect their body weights or lung relative weights. Using ATOR, a prior investigation on mice with lung cancer brought on by benzo(a)pyrene found no differences in lung weights whereas a prior study found no appreciable differences between control and fresh lung or body weights (Liu et al., 2015; Du et al., 2021). The current investigation has also revealed notable alterations in the histological architecture of the lungs, which may be explained by the toxic effects of the Ure+BHT treatment and the increased cellular invasion of the pulmonary tissues. BHT is a substance that promotes tumor growth, and ure is an initiating mutagen for lung cancer. According to the histopathological picture of a normal mouse lung, which is characterized by the epithelium of alveolar ducts and alveoli, BHT undergoes pulmonary metabolism in mice, causing pulmonary inflammation and necrosis of alveolar type-I epithelial cells, with compensatory hyperplasia of alveolar type-II cells, which are the source of lung adenomas. The alterations noted in several stromal cell groups during Ure-induced lung tumorigenesis may result from the tumor's development, which modifies the phenotypes of surrounding niche cells in an apparent attempt to provide a more conducive growth environment (Balli et al., 2012).

Treatment with BHT resulted in increased numbers of infiltrating macrophages and their expression, as well as persistent chronic lung inflammation (Bauer et al., 2001). Reactive oxygen species (ROS) produced by macrophages during prolonged inflammation cause DNA damage in growing cells through the formation of the mutagenic compound peroxynitrite. ROS causes migration, invasion,

and angiogenesis, which in turn drives tumor growth and metastasis (Balli et al., 2012). Point mutations, deletions, and rearrangements in the genome can result from repeated tissue damage and tissue regeneration in the presence of reactive oxygen species (ROS). In the current study, mice given Ure/BHT treatment developed lung lesions of different sizes and kinds. These included just alveolar epithelium hyperplasia and regenerative hyperplasia. Additionally, there were clear neoplastic lesions such adenomas, adenocarcinomas, and adeno-squamous carcinomas. These findings are consistent with a prior study that found that Ure initiates stage-2 carcinogenesis, and BHT then stimulates the development of started cells (Tanaka et al., 2023).

The treatment with ATOR exerted a significant decrease in the number of lung nodules and tumor sizes when compared to the non-treated cancer group (G2). ATOR was confirmed recently to exert an anti-inflammatory effect accompanied by effectiveness on mouse lung histopathology (Marcianò et al., 2022). The results of this investigation showed that the total apoptotic and necrotic cells were greatly enhanced compared to the positive control. The treatment with ATOR employed a significant induction of early and late apoptotic lung tumor cells. It was previously documented that ATOR-treated A549 lung cancer cells die by necrosis as opposed to apoptosis (Tulbah and Gamal, 2021). Statins were shown to induce cell cycle arrest in the late G1 phase *in vitro* (Altwairgi, 2015), and disrupt cell cycle progression via apoptosis induction in the G2/M phase in lung cancer cells *in vitro* (Tulbah and Gamal, 2021).

5. Conclusions

In conclusion, ATOR therapy decreased the carcinogenesis process and enhanced tumor incidences, multiplicities, and histological alterations in lung cancer. Additionally, ATOR therapy enhanced the percentages of apoptotic lung cancer cells and lowered cellular growth.

Conflicts of interest

There are no conflicts of interest to declarework.

References

Abbas Z, Rehman S, 2018. An Overview of Cancer Treatment Modalities. *Neoplasm*, InTech.

Adams SP, Tsang M, Wright JM, 2015. Lipid-lowering efficacy of atorvastatin. Cochrane Database Sys. Rev. 2015: CD008226.

Altwairgi AK, 2015. Statins are potential anticancerous agents (Review). Oncology Reports. 33:1019–1039.

Amin F, Fathi F, Reiner Ž, Banach M, Sahebkar A, 2021. The role of statins in lung cancer. Arch Med Sci. 18:141–152.

Balli D, Ren X, Chou FS, Cross E, Zhang Y, Kalinichenko VV, Kalin TV, 2012. Foxm1 transcription factor is required for macrophage migration during lung inflammation and tumor formation. Oncogene. 31:3875–3888.

Bauer AK, Dwyer-Nield LD, Hankin JA, Murphy RC, Malkinson AM, 2001. The lung tumor promoter, butylated hydroxytoluene (BHT), causes chronic inflammation in promotion-sensitive BALB/cByJ mice but not in promotion-resistant CXB4 mice. Toxicology. 169: 1-15.

Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, Yeger H, 2017. Combination therapy in combating cancer. Oncotarget. 8: 38022–38043.

Dean PN, Jett JH, 1974. Mathematical analysis of DNA distributions derived from flow microfluorometry. J Cell Biol. 60:523–527.

Du X, Li D, Wang G, Fan Y, Li N, Chai L, Li G, Li J, 2021. Chemoprotective effect of atorvastatin against benzo(a)pyrene-induced lung cancer via the inhibition of oxidative stress and inflammatory parameters. Ann Transl Med. 9:355.

Fırıncı F, Karaman M, Cilaker-Mıcılı S, Bagrıyanık A, Uzuner N, Karaman O, 2014. The effect of atorvastatin on lung histopathology in a murine model of chronic asthma. Allergol Immunopathol (Madr). 42:355–361.

Grabarek BO, Boroń D, Morawiec E, Michalski P, Palazzo-Michalska V, Pach Ł, Dziuk B, Świder M, Zmarzły N, 2021. Crosstalk between statins and cancer prevention and therapy: An update. Pharmaceut (Basel). 14:1220.

Gyamfi J, Kim J, Choi J, 2022. Cancer as a Metabolic Disorder. Int. J. Mol. Sci. 23:1155.

Jones GS, Baldwin DR, 2018. Recent advances in the management of lung cancer. Clin. Med. (Lond). 18(Suppl 2): s41-s46.

Klebe S, Leigh J, Henderson DW, Nurminen M, 2019. Asbestos, Smoking and Lung Cancer: An Update. Int. J. Environ. Res. Public Health. 17: 258.

Kong F, Wang N, Gao F, Liang Y, Li L, Yu M, Zhao L, Zhang D, Jia Y, 2023. The clinical application of atorvastatin in patients with small-cell lung cancer with dyslipidemia. J Cancer Res. Clin. Oncol. 149: 13697–13704.

Lemjabbar-Alaoui H, Hassan OU, Yang YW, Buchanan P, 2015. Lung cancer: Biology and treatment options. Biochimica et biophysica acta. 1856:189–210.

Liu C, Chen H, Hu B, Shi J, Chen Y, Huang K, 2023. New insights into the therapeutic potentials of statins in cancer. Front Pharmacol. 14:1188926.

Marcianò G, Palleria C, Casarella A, Rania V, Basile E, Catarisano L, Vocca C, Bianco L, Pelaia C, Cione E, D'Agostino B, Citraro R, De Sarro G, Gallelli L, 2022. Effect of statins on lung cancer molecular pathways: A possible therapeutic role. Pharmaceutics. 15:589.

Salim EI, Aboueisha SS, Khamis AA, 2022. Balanitoside as a natural adjuvant to gemcitabine in lung cancer experimental model. Nut Cancer. 74:388–3402.

Salim EI, Mahfouz ME, Eltonouby EA, Hanafy NA, Hafez EH, 2023. Based polymer nanoparticles from bee pollen attenuate non-small lung cancer through enhancement of apoptosis and cell cycle arrest *in vivo*. Cancer Nanotechnol. 14: 77.

Shehata SA, Toraih EA, Ismail EA, Hagras AM, Elmorsy E, Fawzy MS, 2023. Vaping, Environmental Toxicants Exposure, and Lung Cancer Risk. Cancers. 15: 4525.

Siddiqui F, Vaqar S, Siddiqui AH, 2023. Lung Cancer.

Tanaka R, Yoshinouchi S, Karouji K, Tanaka Y, Tominari T, Hirata M, Matsumoto C, Itoh Y, Miyaura C, Inada M, 2023. A mouse model of lung cancer induced via intranasal injection for anticancer drug screening and evaluation of pathology. FEBS Open Bio. 13(1):51-59.

Tulbah AS, GamalA, 2021. Design and characterization of atorvastatin dry powder formulation as a potential lung cancer treatment. Saudi Pharm. J. 29: 1449–1457.

Zhang Q, Dong J, Yu Z, 2020. Pleiotropic use of Statins as non-lipid-lowering drugs. Int J Biol Sci. 16: 2704–2711.