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Research Article

Effects of Melaleuca alternifolia (Tea Tree) Oil and Vitamin E Combination on the Viability of Non-Small Cell Lung Cancer Cells

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Abstract

Aim: This study aims to examine the cytotoxic effects of Melaleuca alternifolia (tea tree) oil and vitamin E combination (TTO/VE) on a non-small cell lung cancer (NSCLC) cell line, A549 and a normal retinal epithelial cell line, ARPE-19.

Materials and Methods: The cells were incubated with various dilutions of TTO/VE for 24 and 48 hours. The MTS assay was used to determine the cell viability. The dilution-response curves were generated using the MTS data.

Results: 1/400, 1/300, 1/200, 1/100 and 1/20 dilutions of TTO showed a dramatic reduction by more than 80 % and 70 % in the viability of the A549 and ARPE-19 cells, respectively. TTO/VE dilutions of 1/750 and 1/500 resulted in a dose-dependent decrease in the viability of A549 cells, whereas ARPE-19 cells were found to be relatively resistant. No significant decrease in cell viability was obtained for both A549 and ARPE-19 cells in TTO dilutions diluted than 1/1000.

Conclusion: The IC50 value (1/500) of TTO/VE for A549 cells were determined. TTO/VE dilutions of 1/500 were found to be cytotoxic to A549 cells but not to ARPE-19 cells. Although further experiments are needed, these promising preliminary results suggest that TTO/VE could be proposed as a potential natural anticancer agent in lung cancer research.

Key Words: NSCLC; Tea tree oil; Vitamin E; Cytotoxicity

Introduction

Lung cancer stands as the foremost cause of cancer-related mortality globally. More than two million new cases are diagnosed each year. The development of complementary therapies in addition to those currently available may be of benefit. Globally, 19.3 million new cancer-related cases and nearly 10 million deaths were reported in 2020. According to the Global and Regional Cancer Incidence and Mortality Projections (GLOBOCAN 2020), the global cancer burden is projected to increase by 47 % over the next two decades due to an increase in risk factors associated with an aging population, globalization and socioeconomic development[1]. The provision of effective complementary cancer therapies may be critical to global cancer control, as cancer is the leading cause of death before the age of 70, and incidence and prevalence rates are increasing significantly in both across developed and developing countries. Antioxidants have been proposed as potential therapeutic agents against cancer[1,2]. Essential oils isolated from plants have been extensively studied in the scientific literature as potential therapeutics for the treatment of many diseases[3]. Lung cancer remains the leading cause of death from cancer worldwide and is responsible for more than 1.6 million deaths annually[4]. This accounts for about 20 % of all cancer deaths[5].

Plants are and will remain a valuable source of bioactive molecules. They possess antioxidant, and anticarcinogenic properties, rendering them promising candidates against many types of cancer. It is important to note that further research is needed to fully understand their potential benefits and any potential risks associated with their use[6,8]. Tea tree oil (TTO) is a volatile oil obtained through steam distillation from Melaleuca alternifolia, an Australian native plant belonging to the Myrtaceae family. Traditionally, this oil has been used for insect bites and many skin infections[9,12]. It has been reported to possess many medicinal properties including antifungal[13], anti-inflammatory[14], anticancer[15] and anesthetic activity[16].

Vitamin E (VE), a potent fat-soluble antioxidant that can limit the generation of reactive oxidative species (ROS), is an important candidate for adjuvant therapy in cancer. VE can affect apoptosis of cancer cells, reduce chemotherapeutic-induced ROS and enhance the therapeutic effects of anti-cancer agents[17,19]. There are also studies showing that it can suppress the proliferation, migration and growth of cancer cells[20]. However, the literature on the anti-tumor properties of VE remains ambiguous, and more research is required. Furthermore, despite its use in traditional medicine, more studies investigating the anti-cancer properties and efficacy of TTO should be carried out.

In this study, human lung cancer cell line, A549, and normal retinal pigment epithelial control cell line, ARPE-19, were used to assess the effect of a commercial TTO and VE combination (TTO/VE) on cell viability.

Materials and Methods

Reagents and Chemicals

The TTO/VE purchased from Derma-E company (Simi Valley, CA, US) contains Melaleuca Alternifolia Leaf Oil 75 %, Tocopheryl Acetate 25 %. RPMI and PBS were purchased from Gibco (Waltham, MA, USA). Fetal bovine serum (FBS) and trypsin/EDTA (0.25 %) were purchased from Thermo-Fisher (Waltham, MA, USA). The supplier of CellTiter 96® AQueous MTS Reagent Powder was Promega (Madison, WI, USA) and methylphenazinium methylsulfate (PMS) was purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany).

Cell Culture

NSCLC cell line, A549, and retinal pigment epithelial cell line, ARPE-19, were kindly provided by Dr. Saliha Eksi (Medical Microbiology Department, RTEU). The cell lines were cultured in RPMI supplemented with heat-inactivated 10% FBS, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. The cells were cultured in a humidified incubator at 37°C with 5% CO_2. Upon reaching 85% confluence, they were detached using Trypsin/EDTA and sub-cultured into 75 cm^2 flasks.

Cell Viability Assay

The MTS assay was employed to evaluate the viability of A549 and ARPE-19 cell lines. Cells were seeded at a density of 1×10^4 cells/well in 96-well plates and left to adhere overnight. Dilutions of TTO/VE was prepared freshly in complete growth medium. TTO/VE was serially diluted with complete medium to achieve concentrations ranging from 1/20 to 1/5000 (v/v). The attached cells were treated with prepared dilutions for 24 and 48h. The growth of cells was quantified by the ability of viable cells to reduce the MTS to formazan product. After each respective incubation duration, the culture medium was aspirated, and the cells were rinsed with phosphate-buffered saline (PBS). Subsequently, 100 µL per well of MTS/PMS solution was dispensed and incubated at 37 °C for 1-4 hours until a brownish formazan product was developed. The optical density was recorded at 492 nm utilizing a plate reader. (Thermo MultiSkan Go, Thermo Fisher Scientific, USA).

Statistical Analysis

GraphPad Prism T-test calculator tool was employed to assess the disparity between the groups. The error bars represent standard deviations of at least three different experiments. Statistical significance was determined at $p< 0.05$.

Results

Cytotoxicity of TTO/VE on A549 lung cancer and ARPE-19 normal epithelial cells was evaluated. Both cell lines were treated with the dilutions of TTO/VE. Initial experiments were performed with a narrow range of dilutions between 1/25 and 1/200 (Figure 1).

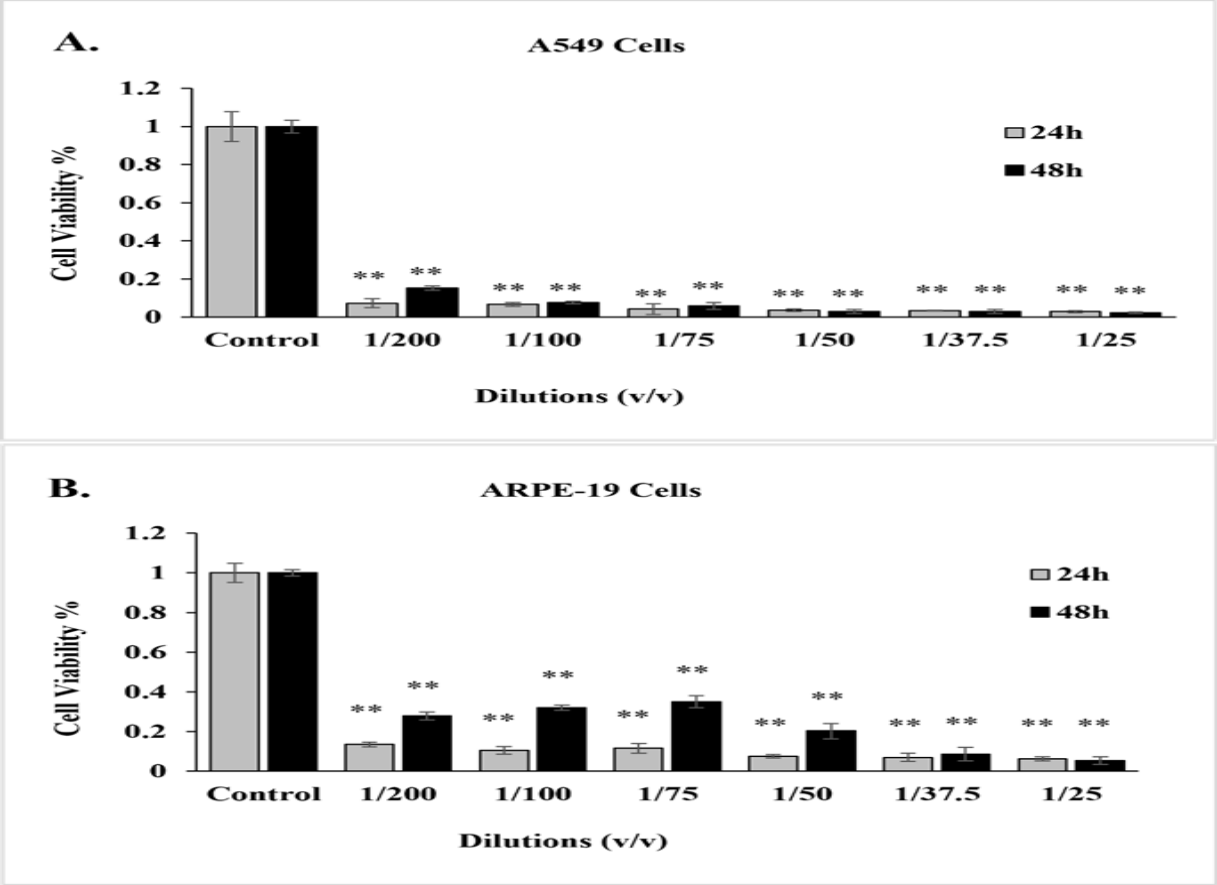

Figure 1. Dilution-response graph for A549 and ARPE-19 cells treated with 1/25-1/200 dilutions of TTO-VE.
Bar graph depiction of cytotoxicity data determined by MTS assay. Values of MTS assay are given in percentage calculated as control group 100 % viable. Asterisk symbol (*) indicates a significant difference to control. Error bars represent standard deviations of at least three different experiments.

A significant cytotoxic effect of TTO/VE was observed on both cell lines. TTO/VE resulted in a 90-95 % decrease in cell viability in all applied dilutions for A549 cells at both 24 and 48h. Although the viability of A549 cells was found to be decreased by 90 % at 24h in all dilutions, a 65-80 % decrease was observed for ARPE-19 cells at 1/50 and lower concentrated dilutions of TTO/VE at 48h. ARPE-19 cells were observed to be relatively resistant to TTO/VE

at 48h. Dilutions between 1/400 and 1/20 of TTO/VE showed a dramatic reduction by more than 90 and 70 % in the viability of the A549 and ARPE-19 cells, respectively (Figure 2).

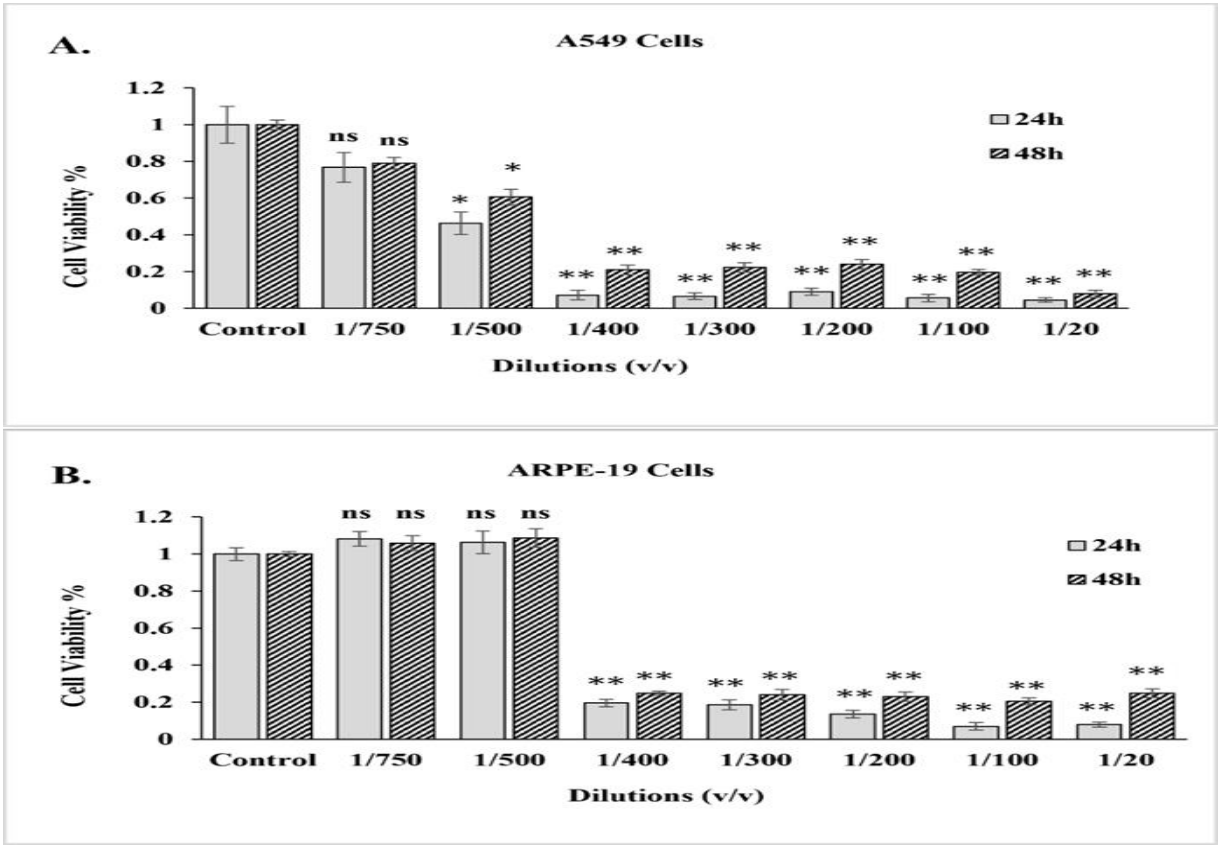

Figure 2. A549 and ARPE-19 cells treated with 1/20-1/750 dilutions of TTO-VE.
Cell proliferation was assessed by the MTS assay. MTS assay values are expressed as percentages. The control group is considered 100 % viable. Asterisk symbol (*) indicates a significant difference to control.

Dose-dependent decrease in the cell viability was observed in 1/750 and 1/500 dilutions of TTO/VE for A549 cells while ARPE-19 cells was found to be relatively resistant at the same dilutions. The viability and proliferation of A549 was significantly inhibited at 1/500 dilution of TTO/VE ($p<0.05$). Inhibitory concentration 50 (IC50) was determined to be 1/500 dilution of TTO/VE for A549 cells at 24h. Further lower concentrated dilutions (1/1000-1/5000) of TTO/VE were also evaluated, shown in Figure 3. There was no statistically significant decrease in cell viability observed for either A549 or ARPE-19 cells across the range of TTO/VE dilutions tested at both time points.

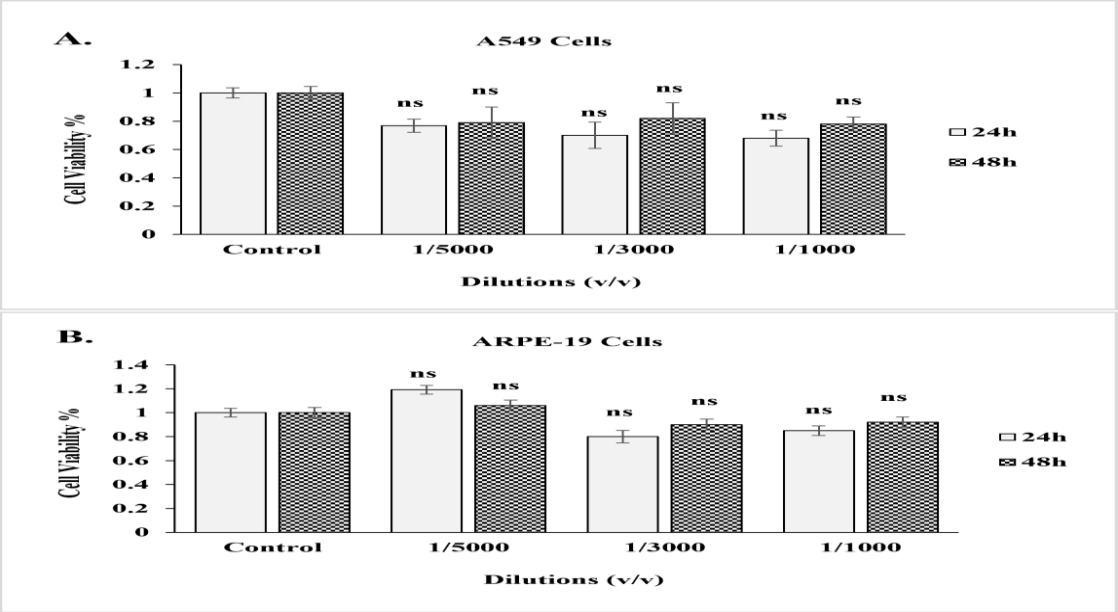

Figure 3. A549 and ARPE-19 cells treated with 1/1000-1/5000 dilutions of TTO-VE
Bar graph represents the percentage of viable cells as determined by MTS assay. cell viability of control group is considered as 100 %. Asterisk (*) represents a significant difference to control.

Discussion

Essential oils are natural, complex mixtures characterized by their strong odor and are formed as secondary metabolites by aromatic plants. Common examples include oils obtained from tea tree, eucalyptus, and peppermint oils. Essential oils consist of 20 to more than 100 different compounds in very different concentrations. Commonly distinguished by their elevated concentrations (ranging from 20% to 70%) of two or three primary constituents, with the remaining compounds typically found in minimal quantities[21,27]. Terpenes represent the most abundant category of secondary metabolites found in essential oils and their effectiveness and impact as anticancer agents have been extensively reported[28,33].

Paclitaxel (Taxol) stands out as one of the most renowned terpene-derived anticancer drugs, with a long history of use in treating various types of cancer[34]. Studies report that Melaleuca-derived oils exhibit in vitro anticancer activity against various human lung, melanoma, liver, and breast cancer cell lines[35,41]. Melaleuca alternifolia is a small tree in the Myrtaceae family native to Australia. TTO produced from the leaves has been utilized for medicinal purposes by Australian tropical medicine[42]. As TTO has a traditional use for a variety of therapeutic indications, there exists a compelling rationale for exploring the effectiveness and safety profile of TTO[43-45]. TTO can be categorized into three main chemotypes: terpinen-4-ol, terpinolene and 1,8-cineole[46]. Terpinen-4-ol functions as a potential anticancer agent and inhibits proinflammatory mediators[47]. In addition to its anticancer properties, TTO has been reported to

show broad-spectrum antimicrobial activity, have anti-fungal and anti-viral effects and increase peripheral blood flow[10,48]. In addition, TTO has demonstrated the ability to inhibit the ability of cells to maintain homeostatic conditions related to the cell growth and replication[10].

In the present study, the cytotoxic effect of TTO/VE on A549 lung cancer cells and ARPE-19 normal epithelial cells has been evaluated. The cells were treated with a wide range of TTO/VE dilutions. This study marks the first investigation in the literature to show the combination of TTO and VE to evaluate cytotoxicity in lung cancer cells. The results show a significant cytotoxic effect on both cell lines at different dilutions of TTO/VE. 1/500 dilution of TTO/VE showed a substantial decrease in cell viability of A549 cells while ARPE-19 cells were relatively resistant to the cytotoxic effects of TTO/VE. The cytotoxic range and IC50 (1/500, 0.2 % [v/v] of TTO/VE) of dilutions for lung cancer cell line was determined. TTO has been reported to exhibit potent cytotoxicity against several cancer cells, including lung cancer (A549), hepatocellular carcinoma (HepG2), breast carcinoma cells (MCF-7), and prostate carcinoma (PC-3)[37-39]. Liu et. al. reported an IC50 of 0.012 % (v/v) dilution for A549 cells which is comparable with our result. The final concentration of TTO (75 %) in the oil extract used in the present study and its combination with VE may explain the difference between the IC50 values. A recent study investigating the anticancer activity of Melaleuca Alternifolia oil extract has reported that the product induced apoptosis and loss of mitochondrial membrane potential in human prostate and breast cancer cell lines[49].

As one of the four fat-soluble vitamins, VE is a very important essential nutrient for human health. As an antioxidant, VE can regulate the production of ROS and modulate signal transduction[50]. Since ROS are involved in many chronic diseases such as cancer and cardiovascular diseases, the possible preventive effects of VE against these diseases have been extensively studied. Low VE intake has been associated with a increased risk of different types of cancer in many studies [51]. The potential anti-cancer properties of VE, which can suppress the proliferation, growth and migration of cancer cells[20], may be attributed to their ability to repress NF-ϰβ and STAT3, two important activators of transcription that are also involved in angiogenesis and metastasis, in addition to promoting apoptosis and cell cycle arrest[52].

VE activity is found in a group of eight fat-soluble compounds consisting of α, β, γ and δ forms of tocopherols (T) and tocotrienols (T3). Alpha-tocopherol (α-T), among the various forms of vitamin E, is the predominant form found in animal tissues and is generally recognized as the 'VE' in the diet[53]. Since α-T is the major form of T found in blood and tissues, it has been extensively employed in cancer prevention studies[54]. However, large-scale human studies with

α-T have yielded conflicting evidence about the anti-cancer activities of T[20,55]. Although randomized controlled trials assessing the cancer prevention potential of VE have shown variable results, recent research indicates a potential inverse association between VE and cancer risk[56]. A nested case-control study examining the association between serum α-T and γ-T levels and the risk of prostate cancer showed that high serum α-T levels reduced the risk of prostate cancer, especially among smokers, while γ-T was not associated with prostate cancer[57]. A similar study also reports that α-T supplementation is significantly associated with a lower incidence of prostate cancer and that higher serum α-T is linked to a decreased risk of developing prostate cancer[58,59]. In addition, numerous observational studies report significant associations between VE and a reduced risk of esophageal, colorectal, lung, pancreatic, kidney and bladder cancer[60-65]. A meta-analysis included data from 6431 individuals showed that colorectal cancer patients had lower serum VE concentrations compared to healthy controls, particularly in European populations[61]. Another meta-analysis study with 575,601 participants from the USA and Europe showed that VE consumption was inversely associated with bladder cancer risk[66]. Chen et al. also found that α-T was associated with a reduced risk of bladder cancer[60]. In two of the three reported cohort studies on lung cancer, there was a significant inverse association between dietary VE intake and lung cancer risk. Of the four case-control studies on lung cancer, three studies found lower serum α-T levels in lung cancer patients than in matched controls[51].

The effect of TTO/VE combination on normal and lung cancer may be less effective for normal epithelial cells and may be considered a potential therapeutic compound in a certain dose range. Furthermore, the potent cytotoxic effect of TTO/VE for lung cancer cells, but not for normal epithelial cells at the same dilution, suggests that its content may have a potential as a candidate cancer therapeutic. It is important to obtain the pure extract of TTO and identify bioactive constituents in the future. In addition, the effect of TTO on lung cancer cells in the presence and absence of VE should be examined in multiple lung cancer cell lines in advanced experimental models. Additional studies are warranted to comprehend the molecular mechanisms that underlie the anticancer activity of TTO/VE.

Conclusion

Our study demonstrates the significant cytotoxic effects of TTO/VE on the NSCLC cell line, A549, and the normal epithelial cell line, ARPE-19. Our findings reveal that TTO/VE at various dilutions exhibits potent anticancer activity against A549 cells, with notable dose-dependent effects observed. However, ARPE-19 cells demonstrate relative resistance to

TTO/VE, suggesting a potential selectivity towards cancer cells. Since the TTO/VE combination has a toxic effect on both cancerous and non-cancerous cells, especially at high doses, it is important to consider the concentration that is non-toxic to non-cancerous cells but toxic to cancerous cells when applying the combination for further studies. Importantly, our results highlight the importance of optimizing the concentration of TTO/VE to achieve maximal therapeutic efficacy while minimizing adverse effects on normal cells. Overall, these findings contribute to the growing body of evidence supporting the potential utility of TTO/VE as a promising candidate for further investigation in the treatment of NSCLC, with careful consideration of its impact on normal cellular physiology. Further research exploring the underlying mechanisms of TTO/VE-induced cytotoxicity and its potential synergistic effects with existing therapeutic agents may provide valuable insights for the development of novel therapeutic strategies against NSCLC.

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