β-Elemene Restrains PTEN mRNA Degradation to Restrain the Growth of Lung Cancer Cells via METTL3-Mediated N6 Methyladenosine Modification

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Received 18 October 2021; Revised 19 November 2021; Accepted 24 November 2021; Published 12 January 2022

Academic Editor: Qin Yuan

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Lung cancer is one of the most fatal malignancies and the leading cause of cancer death worldwide. β-Elemene, a well-known anticancer drug, has drawn a great deal of attention from researchers attributed to its limited side impacts. N6-Methyladenosine (m6A) modification is the most common RNA modification and plays a vital role in the pathogenesis of multiple tumors. However, the functional link between β-elemene and the m6A modification in lung cancer development remains unexplored. In this study, we investigated whether m6A modification was responsible for the impacts of β-elemene on lung cancer. Firstly, outcomes suggested that β-elemene restrained the malignant behaviors of A549 togethertogether with H1299 cells. Thereafter, we observed that β-elemene markedly regulated METTL3, YTHDF1, and YTHDC1 among various m6A modulators. METTL3 was selected for further study because of its oncogenic function in lung cancer. RT-qPCR and western blot assays exhibited that the mRNA and protein expression levels of METTL3 were lessened by the administration of β-elemene. Mechanistically, β-elemene exerted the restrictive impacts on the cell growth of lung cancer in vivo and in vitro through targeting METTL3. More importantly, β-elemene contributed to the augmented PTEN expression via suppressing its m6A modification. To sum up, we provided strong clues that β-elemene promoted PTEN expression to retard lung cancer progression by the regulation of METTL3-mediated m6A modification.

1. Introduction

Lung cancer is one of the most prevailing malignant tumors around the world, and its morbidity and mortality rank first among all the cancers [1, 2]. As a fatal disease, lung cancer seriously endangers human life and aggravates the global public health burden [3]. The deaths from lung cancer are approximately 1.8 million in 2018, and it is estimated that the number of cases succumbed to lung cancer will rise to 3 million by 2035 worldwide [4, 5]. The therapeutic interventions for lung cancer mainly consist of surgical resection, radiotherapy, chemotherapy, and gene-targeted therapy, and surgery resection is a radical therapy in the absence of metastasis [6, 7]. The overall 5-year survival rate is still stagnant at about 15% in spite of tremendous advance in techniques and therapeutic methods [8]. Hence, characterizing the molecular mechanism governing lung cancer progression and exploring effective targets are urgently needed to improve the administration of lung cancer.

Traditional Chinese medical herb Curcuma zedoaria, also termed as Rhizoma zedoariae, is a member of the Zingiberaceae family, which is widely employed to treat multiple disorders, including cancer [9–11]. Elemene is a primary constituent of the essential oil of Curcuma zedoaria and is categorized into α, β, δ, and γ-elemene [12]. Moreover, β-elemene, as the major active ingredient segregated
from Curcuma zedoaria, has been proven to be a non-cytotoxic anticancer agent in a wide range of malignancies, such as renal cell carcinoma, breast cancer, glioma, and gastric cancer [13–16]. Mechanically, β-elemene leads to cancer cell cycle arrest, facilitates cancer cell apoptosis, restrains the resistance of tumor cells to chemotherapy and radiotherapy without myelosuppression, heightens the immunogenicity of cancer cells, and exhibits less side impacts than other drugs with significant liver and kidney damage [17–19]. In recent years, there has been increasing interest in seeking potential function and regulatory mechanism of β-elemene in human cancer. Although β-elemene has been demonstrated to present anticancer impacts on the development of lung cancer [20], more specific mechanisms are largely to be further expounded.

Mounting evidence indicates that epigenetic modification plays a crucial role in cellular activities and the development of various diseases [21, 22]. N°-Methyladenosine (m°A) modification is the most ubiquitous chemical modification of RNAs and is a dynamic and invertible process controlled by methyltransferases (known as “writers”) like methyltransferase-like 3/14 (METTL3/14) and Wilms tumor 1-associated protein (WTAP), as well as demethylases (named as “erasers”) such as alkylation repair homolog protein 5 (ALKBH5) and fat-mass and obesity-associated protein 5 (ALBKH5) [23]. Accumulating studies emphasize that m°A modification is participated in tumor progression by regulating diverse biological processes, including embryonic development [24], immunity [25], and metabolism [26]. However, the relationship between β-elemene and m°A modification has never been investigated.

The purpose of this study is to validate the function of β-elemene in cell expansion and apoptosis of lung cancer and elucidate its molecular mechanism, which provides novel insights into the potential role of m°A modification in the impacts of β-elemene on lung cancer.

2. Materials and Methods

2.1. Cell Culture. Two human lung cancer cell lines A549 and H1299 were supplied by American Type Culture Collection (ATCC, Manassas, USA) and maintained in DMEM (Gibco, USA) containing 10% fetal bovine serum and 1% penicillin/streptomycin. The two cell lines were cultured in a humid incubator at 37°C in the presence of 5% CO₂.

2.2. Cell Administration and Transfection. For the administration of β-elemene [27–29], A549 together with H1299 cells were treated with 0, 10, 50, and 100 μg/ml β-elemene acquired from Dalian Holley Jingang Pharmaceutical Co., Ltd. (China) for 24 h. To upregulate METTL3 expression, the pcDNA3.1 plasmids expressing METTL3 named as pcDNA3.1/METTL3 were designed and generated by Synbio Technologies (China). The empty vector served as the negative control. A549 together with H1299 cells were transfected with the indicated plasmids by utilizing Lipofectamine 2000™ (Invitrogen, USA) according to the product instructions.

2.3. Reverse Transcription-Quantitative PCR (RT-qPCR). Total RNA from A549 together with H1299 cells was isolated by TRIzol reagent and then reversed-transcribed into cDNA with Prime-Script RT Master Mix kit (Takara, Japan) obeying the vendor’s directions. Subsequently, the PCR assay was conducted on an Applied Biosystems 7500 PCR Detection System by utilizing a SYBR Premix Ex Taq Kit (TaKaRa, China). The sequences of main primers implemented were listed as follows: the primers of METTL3: 5′-AAGCAGCATTCAAGAAT-3′ (sense) and 5′-GGAATCACCTCGACACCTC-3′ (antisense); PTEN primers: 5′-TCCAGACATGACAGCCATC-3′ (sense) and 5′-TGCTTTGAATCCTAAACACTTACT-3′ (antisense); the primers for β-actin: 5′-ACTGGAAAGCTGAAGGTA-3′ (sense) and 5′-AGAGAAGTGCTGGCTTTT-3′ (antisense). The gene expression level was calculated by the relative 2-ΔΔCt method, and β-actin was an endogenous control for normalization.

2.4. Cell Proliferation Assay. Cell Counting Kit-8 (CCK-8) assay was employed for the estimation of cell expansion. A549 together with H1299 cells were harvested after different administrations and seeded into a 96-well plate at a density of 5 × 10⁴ cells per well. At 0, 24, 48, and 72 h post-incubation at 37°C, each well was supplemented with 10 μl of CCK-8 reagent and then A549 together with H1299 cells underwent additional 4 h of incubation at 37°C. The absorbance at 450 nm was determined by a microplate reader (Life Science Co., China).

2.5. Transwell. After the cells were routinely transfected for 24 hours, the cells were digested and centrifuged. And the cells were reseeded into the top of the insert of a Boyden chamber (Corning Inc., Corning, NY, USA) with 300 μg/mL Matrigel. After 20–24 h incubation, invasive cells that passed through the filter were fixed with 0.1% paraformaldehyde (Solarbio Science & Technology Co., Ltd., Beijing, China) and stained with 0.1% crystal violet solution. Finally, place the chamber under a microscope to observe and take pictures. For the transwell migration assay, all procedures were similar but without the incubation of Matrigel.

2.6. Flow Cytometry. Flow cytometry analysis was performed to measure cell apoptosis with Annexin V and FITC Apoptosis Detection Kit (BD Bioscience, USA). In short, A549 together with H1299 cells were trypsinized, collected, and rinsed twice by using PBS following 24 h of β-elemene administration. Thereafter, cells were stained by 5 μL Annexin V-FITC and 5 μL PI in the dark in accordance with the manufacturer’s recommendations. The apoptosis of A549 together with H1299 cells was explored with flow cytometry and CellQuest™ Pro software (BD Biosciences).

2.7. Western Blot. Total protein extraction was carried out with RIPA lysis buffer (Beyotime, China), and protein concentration was checked by a BCA kit (Beyotime) based on the manufacturer’s protocols. Protein samples were...
detached on 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, USA). After blockage in 5% defatted milk, membranes were probed by primary antibodies for METTL3 and GAPDH (all obtained from Abcam, USA) at 4°C all the night, followed by incubation with appropriate secondary antibodies at room temperature for 2 h, and examined by an ECL detection system (Eastman Kodak, USA). The bands were quantified with Image Quant software, and GAPDH was implemented as the inherent reference.

2.8. Detection of the m6A Level. After extraction with TRIzol (Invitrogen), total RNA was purified by GenElute™ mRNA Miniprep Kit (Sigma, USA) in line with the instructions recommended by the supplier. Then, the total level of m6A in mRNA was examined with EpiQuik m6A RNA Methylation Quantification Kit (EpiGentek, USA) according to the manufacturer’s protocols; 200 ng of poly-A-purified RNAs was loaded on assay wells, followed by the addition of capture antibody solution and detection antibody reagent. Lastly, the m6A level was colorimetrically checked at 450 nm and therewith calculated with the standard curve.

2.9. m6A Immunoprecipitation (MeRIP). The m6A modification of PTEN gene was measured with the MeRIP-PCR assay. In briefly, A549 together with H1299 cells were subjected to RNA extraction by TRIzol and purified with the Dynabeads™ mRNA Purification Kit (Invitrogen) according to the product manuals. Cell extracts were incubated with Pierce™ Protein A/G Magnetic Beads pretreated with anti-m6A antibody (Millipore) or negative control IgG (Millipore) at 4°C for 2 h. m6A-Modified RNAs were eluted from the beads using proteinase K and elution buffer, and PTEN in precipitates was explored by the RT-qPCR assay.

2.10. Animals Experiments. Four-week-old BALB/c nude mice were randomly divided into three groups: (1) vector group, (2) vector + β-elemene group, and (3) β-elemene + METTL3 group. Nude mice were raised in an SPF level animal house and were free to eat and drink. Mice in the vector group were subcutaneously injected with lung cancer cells transfected with empty vector and did not receive β-elemene administration, and this group was implemented as the negative control. Following establishing orthotopic xenografts by using A549 or H1299 cells transfected with empty vector, mice in the vector + β-elemene group underwent intraperitoneal injection with β-elemene once a day. For the subcutaneous transplanted model, A549 or H1299 cells transfected with METTL3-overexpressing vector were inoculated into mice from the β-elemene + METTL3 group. Then, mice were intraperitoneally administrated with β-elemene once a day. Three weeks later, all the animals were euthanized with CO2. Xenografts were removed and weighted after mice were euthanized. The volume of neoplasms was monitored once a week. The experiment was approved by Animal Ethics Committee of Jiangsu Cancer Hospital (No. 2018-0012).

2.11. TUNEL Staining. The TUNEL assay was performed according to the instructions provided by Vanzyme (A111, Nanjing, China). The images were acquired by fluorescence microscopy (IX61, Olympus, Tokyo, Japan).

2.12. Statistical Analysis. All experimental outcomes were reported as mean ± standard deviation (SD), and each assay was repeated at least three times. Statistical analyses were implemented with SPSS 16.0 software. Differences between two groups were assessed by Student’s t-test, and one-way ANOVA followed by the Bonferroni test was utilized for comparisons among multiple groups. P value <0.05 was set as statistically significant.

3. Results

3.1. β-Elemene Suppressed Cell Expansion and Induced the Apoptosis of Lung Cancer Cells. In order to verify the regulatory role of β-elemene in lung cancer progression, A549 together with H1299 cells were firstly exposed to different concentrations of β-elemene (10, 50, and 100 μg/ml) and then estimated with the CCK-8 assay and flow cytometry analysis. As demonstrated in Figure 1(a), β-elemene prominently weakened lung cancer cell expansion in a dose-dependent manner. In consistent with the foregoing outcomes, we observed that the apoptosis rate of A549 together with H1299 cells was gradually decreased owing to the increase of β-elemene concentration (Figures 2(a) and 2(b)). In a word, these findings revealed the antitumor impacts of β-elemene on lung cancer.

3.2. β-Elemene Contributed to the Decreased METTL3 in Lung Cancer Cells. Subsequently, we intended to elaborate the molecular mechanism of β-elemene by exploring the relationship between β-elemene and m6A modification. The RT-qPCR assay was carried out to determine the function of β-elemene in the expression levels of m6A modulators, including methyltransferases METTL3, METTL14, and WTAP, and demethylases FTO and ALKBH5, as well as m6A-binding proteins YTHDF1 and YTHDC1. Outcomes indicated that β-elemene administration led to the decreased expression of METTL3 and YTHDF1, whereas the augment of YTHDC1 level was upregulated (Figure 3(a)). Considering that the important role of METTL3 in lung cancer has been reported, METTL3 was chosen for the in-depth study. RT-qPCR analysis and western blot delineated that METTL3 expression was reduced in β-elemene-induced lung cancer cells at both mRNA and protein levels (Figures 3(b)-(d)). Collectively, β-elemene restrained the METTL3 level.

3.3. Overexpression of METTL3 Abrogated the Regulatory Role of β-Elemene in Lung Cancer Progression. In Figure 4(a), we provided qRT-PCR detection results for the transfection efficiency of METTL3. To confirm whether METTL3 mediated the impacts of β-elemene on lung cancer, we performed the rescue experiments. The data from the RT-qPCR assay disclosed that the descended level of METTL3 caused...
β-elemene was recovered by enhanced expression of METTL3 (Figure 4(b)). The CCK-8 assay suggested that METTL3 upregulation abolished the impacts of β-elemene administration on the viability of A549 together with H1299 cells (Figure 4(c)). Concordantly, the promotion of cell apoptosis in β-elemene-induced lung cancer cells was counteracted by the overexpression of METTL3 (Figures 4(d)–4(e)). Taken together, we concluded that β-elemene exhibited anticancer activities in lung cancer via the modulation of METTL3.

3.4. β-Elemene Executed Restrictive Impacts on the Growth of Lung Cancer Cells In Vivo by Targeting METTL3.

Therewith, we further validated the role of β-elemene/METTL3 axis in lung cancer in vivo by conducting xenograft experiments. Our observations exhibited that the size of tumors formed by nude mice administrated with β-elemene was smaller than that in the matched group, whereas tumor size was augmented when mice were injected with METTL3-overexpressing A549 together with H1299 cells followed by β-elemene administration (Figure 5(a)). The weight and volume of neoplasms in the β-elemene group were lower compared with those in the matched group, and the overexpression of METTL3 reversed tumor growth in mice treated with β-elemene (Figures 5(b)–5(d)). TUNEL staining was performed to verify the apoptosis rate of different groups. And the results indicated that β-elemene promoted the apoptosis of lung cancer tissue, whereas overexpressing METTL3 reversed the effect induced by β-elemene (Figures 5(e) and 5(f)). β-Elemene increased the expression of Bax and caspase 3, but downregulated the expression of Bcl-2. But overexpressing METTL3 inhibits the effect of β-elemene (Figures 5(f)–5(g)). On the whole, β-elemene restrained cell growth in lung cancer in vivo through repressing METTL3 expression.
3.5. β-Elemene Enhanced PTEN Expression through Restraining METTL3-Mediated m^6^A Modification.

In view of the fact that PTEN is a vital tumor suppressor in lung cancer and METTL3 serves as a mediator in regulating PTEN expression, we investigated the m^6^A level in lung cancer cells after β-elemene administration. Our findings suggested that β-elemene significantly declined the level of m^6^A methylation in lung cancer cells (Figure 6(a)). On the contrary, PTEN expression was overtly elevated in A549 together with H1299 cells due to the administration of β-elemene (Figure 6(b)). Importantly, the upregulation of METTL3 resulted in the restoration of the PTEN expression level in β-elemene-treated lung cancer cells (Figure 6(c)). As shown in Figure 6(d), the forced expression of METTL3 exerted a restrictive role in the PTEN level. Moreover, we indicated that METTL3 overexpression promoted the m^6^A modification of PTEN, whereas β-elemene produced the opposite result (Figure 6(c)). Namely, β-elemene protected PTEN from METTL3-mediated m^6^A modification.

4. Discussion

Lung cancer is regarded as the deadliest cancer and the leading contributor of cancer-associated deaths throughout the world [30]. The prevalence of lung cancer in the elderly is staggering during recent decades, and the demographic shift is responsible for the rising risk of cancer [31]. Of note, lung cancer becomes a health impediment for the public on account of its highest incidence and death rates in all the malignant tumors [32, 33]. Additionally, due to the lack of the potent biomarkers in the diagnosis and therapy of lung cancer, its 5-year survival rate remains far from satisfactory for all stages [34]. In view of these facts, it is indispensable to identify effective therapeutic strategies for lung cancer administration.

As an active compound derived from Curcuma zedoaria, β-elemene exerts antitumor activities in a variety of malignant tumors, including lung cancer [27, 35, 36]. For instance, β-elemene attenuates peritoneal metastasis in gastric
β-Elemene impedes the progression of bladder cancer by upregulating PTEN and restraining AKT phosphorylation [38]. β-Elemene enhances the radiosensitivity of lung cancer A549 cells through promoting DNA damage and suppressing DNA repair [39]. Herein, we treated lung cancer cells with different concentrations of β-elemene to confirm the anticancer role of β-elemene in lung cancer. Outcomes of the CCK-8 assay and flow cytometry suggested that β-elemene repressed cell expansion and induced cell apoptosis in a dose-dependent manner.

Nevertheless, the exact mechanism underlying β-elemene in lung cancer is still not fully understood despite plentiful research studies on this subject in recent years. A growing number of explorations demonstrate that m6A modification plays a regulatory role in tumor development [40]. Importantly, m6A modification modulators, including methyltransferases, demethylases, and binding proteins, are involved in the initiation and evolution of human cancer by numerous mechanisms, such as regulating mRNA splicing and stability, controlling nuclear export, affecting translation efficiency, and mediating microRNA processing [41–45]. Furthermore, METTL3, a well-known m6A modification “writer,” is of great significance in the regulation of m6A modification and functions as a critical mediator in the tumorigenesis and progression of lung cancer [46]. Increasing evidence reveals that METTL3 is highly expressed and exhibits oncogenic properties in lung cancer [47, 48]. In this study, we found that the mRNA and protein expression of METTL3 was going lower with the increase of β-elemene dose. Furthermore, the upregulation of METTL3 reversed the viability and apoptosis of β-elemene-induced lung cancer cells both in vivo and in vitro.

It is extensively accepted that phosphatase and tensin homolog (PTEN) serves as a tumor suppressor gene in the progress of multiple malignancies, including lung cancer [49, 50]. Additionally, low expression of PTEN is strongly correlated with the poor prognosis of patients with lung cancer [51]. And more notably, METTL3 has been reported to regulate the stability of PTEN via the m6A mechanism [52]. Our experimental data ulteriorly unraveled that m6A modification was lessened and PTEN level was augmented in β-elemene-treated lung cancer cells. Moreover, METTL3 contributed to the decrease of PTEN expression. Finally, we validated the restrictive impacts of β-elemene on the malignant behaviors of lung cancer cells, which were mediated by the METTL3-regulated m6A modification of PTEN. This study found that β-elemene plays an antitumor role by inducing iron death in lung cancer cells. β-Elemene has a certain development potential, but it needs to be further studied as an antitumor drug.
Figure 4: Continued.
Figure 4: Overexpression of METTL3 abrogated the regulatory role of β-elemene in lung cancer progression. (a) qRT-PCR detection results for the transfection efficiency of METTL3. (b) The efficacy of METTL3 overexpression was verified by RT-qPCR analysis. (c) CCK-8 assay was applied to detect the viability of A549 together with H1299 cells after different administrations. (d) The role of β-elemene/METTL3 in lung cancer cell apoptosis was evaluated by flow cytometry. (e) The quantitation of flow cytometry outcomes. Experimental data were represented as mean ± SD, and all assays were repeated thrice.

Figure 5: Continued.
Figure 5: β-Elemene executed restrictive impacts on the growth of lung cancer cells in vivo by targeting METTL3. (a) The images of neoplasms from three groups: (1) vector group; (2) vector + β-elemene group; (3) β-elemene + METTL3 group. (b) The weight of xenografts formed by nude mice in indicated groups. (c, d) The volume of tumors formed by nude mice with different administrations. (e) TUNEL staining of lung cancer tissue of different groups. (f-h) The expressions of Bax, Bcl-2, and caspase 3 in the tissues of different groups. Experimental data were represented as mean ± SD, and all assays were repeated thrice.

Figure 6: β-Elemene enhanced PTEN expression through restraining METTL3-mediated m⁶A modification. (a) The detection outcomes of the total m⁶A level in A549 and H1299 cells administrated with β-elemene. (b, d) The RT-qPCR assay was adopted to examine PTEN in A549 together with H1299 cells following different administrations. (e, f) The impacts of β-elemene and METTL3 on the m⁶A modification of PTEN mRNA were estimated with the MeRIP-PCR assay. The experimental data were represented as mean ± SD, and all assays were repeated thrice.
In conclusion, the current study shed light on the association between β-elemene and m^6A modification for the first time. We illuminated that β-elemene acted as an anticancer agent in lung cancer via the regulation of METTL3-mediated PTEN pathway in a m^6A manner, which disclosed a novel mechanism of β-elemene suppressing lung cancer development and provided convincing evidence supporting β-elemene as a potent for the administration of lung cancer.

**Data Availability**

The analyzed datasets generated during the study are available from the corresponding author on reasonable request.

**Ethical Approval**

The experiment was approved by the Animal Ethics Committee of Jiangsu Cancer Hospital.

**Consent**

It is not applicable.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Yuxu Feng and Chenchen Li authors contributed equally to this work.

**Acknowledgments**

The project was supported by the Cadre Health Research Project of Jiangsu Province (Grant/Award Number: B118033).

**References**

[1] J. S. J. Lim and R. A. Soo, “Nivolumab in the treatment of metastatic squamous non-small cell lung cancer: a review of the evidence,” *Therapeutic Advances in Respiratory Disease*, vol. 10, no. 5, pp. 444–454, 2016.

[2] A. Jemal, F. Bray, M. M. Center, J. Ferlay, E. Ward, and D. Forman, “Global cancer statistics,” *CA: A Cancer Journal for Clinicians*, vol. 61, no. 2, pp. 69–90, 2011.

[3] J. Wang, X. Liu, X. Peng, and G. Ding, “miR-142-5p regulates CD4+ T cells in human non-small cell lung cancer through PD-L1 expression via the PTEN pathway,” *Oncology Reports*, vol. 40, no. 1, pp. 272–282, 2018.

[4] J. Ferlay, M. Colombet, I. Soerjomataram et al., “Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods,” *International Journal of Cancer*, vol. 144, no. 8, pp. 1941–1953, 2019.

[5] J. Didikowska, U. Wojciechowska, M. Mańczuk, and J. Łobaszewski, “Lung cancer epidemiology: contemporary and future challenges worldwide,” *Annals of Translational Medicine*, vol. 4, no. 8, p. 150, 2016.

[6] J. G. Shi, H. J. Shao, F. E. Jiang, and Y. D. Huang, “Role of radiation therapy in lung cancer management - a review,” *European Review for Medical and Pharmacological Sciences*, vol. 20, no. 15, pp. 3217–3222, 2016.

[7] J. Köhler, “Second-line treatment of NSCLC-the pan-ErbB inhibitor afatinib in times of shifting paradigms,” *Frontiers of Medicine*, vol. 4, p. 9, 2017.

[8] P. Martin and N. B. Leighl, “Review of the use of pretest probability for molecular testing in non-small cell lung cancer and overview of new mutations that may affect clinical practice,” *Therapeutic Advances in Medical Oncology*, vol. 9, no. 6, pp. 405–414, 2017.

[9] H. Matsuda, K. Ninomiya, T. Morikawa, and M. Yoshikawa, “Inhibitory effect and action mechanism of sesquiterpenes from zedoariae rhizoma on d-galactosamine/lipopolysaccharide-induced liver injury,” *Bioorganic & Medicinal Chemistry Letters*, vol. 8, no. 4, pp. 339–344, 1998.

[10] S. Lakshmi, G. Padmaja, and P. Remani, “Antitumour effects of isolucumeneol isolated from *Curcuma zedoaria* rhizomes on human and murine cancer cells,” *International Journal of Medicinal Chemistry*, vol. 2011, Article ID 253962, 13 pages, 2011.

[11] J. S. Jurenka, “Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research,” *Alternative Medicine Review: A Journal of Clinical Therapeutic*, vol. 14, no. 2, pp. 141–153, 2009.

[12] A. Edris, “Anti-cancer properties of nigella spp. essential oils and their major constituents, thymoquinone and β-elemene,” *Current Clinical Pharmacology*, vol. 4, no. 1, pp. 43–46, 2009.

[13] Y.-H. Zhan, J. Liu, X.-J. Qu et al., “β-elemene induces apoptosis in human renal-cell carcinoma 786-0 cells through inhibition of MAPK/ERK and PI3K/Akt/mTOR signalling pathways,” *Asian Pacific Journal of Cancer Prevention*, vol. 13, no. 6, pp. 2739–2744, 2012.

[14] L.-j. Chen, X. Zheng, Y.-p. Shen et al., “Higher numbers of T-bet+ intratumoral lymphoid cells correlate with better survival in gastric cancer,” *Cancer Immunology, Immunotherapy*, vol. 62, no. 3, pp. 553–561, 2013.

[15] Y.-Q. Yao, X. Ding, Y.-C. Jia, C.-X. Huang, Y.-Z. Wang, and Y.-H. Xu, “Anti-tumor effect of β-elemene in glioblastoma cells depends on p38 MAPK activation,” *Cancer Letters*, vol. 264, no. 1, pp. 127–134, 2008.

[16] J. Liu, Y. Zhang, J. Qu et al., “β-Elemene-induced autophagy protects human gastric cancer cells from undergoing apoptosis,” *BMC Cancer*, vol. 11, no. 1, p. 183, 2011.

[17] B. Zhai, Y. Zeng, Z. Zeng et al., “Drug delivery systems for elemene, its main active ingredient β-elemene, and its derivatives in cancer therapy,” *International Journal of Nano-Medicine*, vol. 13, pp. 6279–6296, 2018.

[18] S. Liu, L. Zhou, Y. Zhao, and Y. Yuan, “β-elemene enhances both radiosensitivity and chemosensitivity of glioblastoma cells through the inhibition of the ATM signaling pathway,” *Oncology Reports*, vol. 34, no. 2, pp. 943–951, 2015.

[19] J. Wang, H. Zhang, and Y. Sun, “Phase III clinical trial of elemenum emulsion in the management of malignant pleural and peritoneal effusions,” *Zhonghua Zhongliu Zazhi*, vol. 18, no. 6, pp. 464–467, 1996.

[20] Z. Wu, T. Wang, Y. Zhang et al., “Anticancer effects of β-elemene with hyperthermia in lung cancer cells,” *Experimental and Therapeutic Medicine*, vol. 13, no. 6, pp. 3153–3157, 2017.

[21] B. Yao, K. M. Christian, C. He, P. Jin, G.-L. Ming, and H. Song, “Epigenetic mechanisms in neurogenesis,” *Nature Reviews Neuroscience*, vol. 17, no. 9, pp. 537–549, 2016.

[22] M. Esteller, “Cancer epigenomics: DNA methylomes and histone-modification maps,” *Nature Reviews Genetics*, vol. 8, no. 4, pp. 286–298, 2007.
[23] B. Yue, C. Song, L. Yang et al., “METTL3-mediated N6-methyladenosine modification is critical for epithelial-mesenchymal transition and metastasis of gastric cancer,” *Molecular Cancer*, vol. 18, no. 1, p. 142, 2019.

[24] S. Geula, S. Moshitch-Moshkovitz, D. Dominissini et al., “m6A mRNA methylation facilitates resolution of naïve pluripotency toward differentiation,” *Science*, vol. 347, no. 6225, pp. 1002–1006, 2015.

[25] X. Zhong, J. Yu, X. Hu, and X. Cao, “The RNA helicase DDX46 inhibits innate immunity by entrapping m6A-demethylated antiviral transcripts in the nucleus,” *Nature Immunology*, vol. 18, no. 10, pp. 1094–1103, 2017.

[26] B. Yue, C. Song, L. Yang et al., “β-Elemene: mechanistic studies on cancer cell interaction and its chemosensitization effect,” *Frontiers in Pharmacology*, vol. 08, p. 105, 2017.

[27] Q. Li, G. Wang, F. Huang, M. Banda, and E. Reed, “Antineoplastic effect of β-elemene on prostate cancer cells and other types of solid tumour cells,” *Journal of Pharmacy and Pharmacology*, vol. 62, no. 8, pp. 1018–1027, 2010.

[28] B. Zhai, N. Zhang, X. Han et al., “Molecular targets of β-elemene, a herbal extract used in traditional Chinese medicine, and its potential role in cancer therapy: a review,” *Biomedicine & Pharmacotherapy*, vol. 114, Article ID 108812, 2019.

[29] F. Bray, J. Ferlay, I. Soerjomataram, P. D. P. D. B. Baade, D. R. Youlden, R. L. Siegel, K. D. Miller, and A. Jemal, “Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries,” *CA: A Cancer Journal for Clinicians*, vol. 68, no. 6, pp. 394–424, 2018.

[30] P. M. de Groot, C. C. Wu, B. W. Carter, and R. F. Munden, “The epidemiology of lung cancer,” *Translational Lung Cancer Research*, vol. 7, no. 3, pp. 220–233, 2018.

[31] R. L. Siegel, K. D. Miller, and A. Jemal, “Cancer statistics, 2018,” *CA: A Cancer Journal for Clinicians*, vol. 68, no. 1, pp. 7–30, 2018.

[32] T.-Y. D. Cheng, S. M. Cramb, P. D. Baade, D. R. Youlden, C. Nwogu, and M. E. Reid, “The international epidemiology of lung cancer: latest trends, disparities, and tumor characteristics,” *Journal of Thoracic Oncology*, vol. 11, no. 10, pp. 1653–1671, 2016.

[33] E. Shitivelman, T. Hensing, G. R. Simon et al., “Molecular pathways and therapeutic targets in lung cancer,” *Oncotarget*, vol. 5, no. 6, pp. 1392–1433, 2014.

[34] W. Tan, J. Lu, M. Huang et al., “Anti-cancer natural products isolated from Chinese medicinal herbs,” *Chinese Medicine*, vol. 6, no. 1, p. 27, 2011.

[35] S. Zhao, J. Wu, F. Zheng et al., “β-elemene inhibited expression of DNA methyltransferase 1 through activation of ERK 1/2 and AMPK α signaling pathways in human lung cancer cells: the role of Sp1,” *Journal of Cellular and Molecular Medicine*, vol. 19, no. 3, pp. 630–641, 2015.

[36] M. Deng, Y. Zhang, B. Liu et al., “β-elemene inhibits peritoneal metastasis of gastric cancer cells by modulating FAK/Claudin-1 signaling.” *Phytotherapy Research*, vol. 33, no. 9, pp. 2448–2456, 2019.

[37] B. Cai, L. Ma, S. Nong, Y. Wu, X. Guo, and J. Pu, “β-elemene induced anticancer effect in bladder cancer through upregulation of PTEN and suppression of AKT phosphorylation,” *Oncology letters*, vol. 16, no. 5, pp. 6019–6025, 2018.

[38] L.-J. Li, L.-F. Zhong, L.-P. Jiang, C.-Y. Geng, and L.-I. Zou, “β-elemene radiosensitizes lung cancer A549 cells by enhancing DNA damage and inhibiting DNA repair,” *Phytotherapy Research*, vol. 25, no. 7, pp. 1095–1097, 2011.

[39] X. Deng, R. Su, X. Feng, M. Wei, and J. Chen, “Role of N6-methyladenosine modification in cancer,” *Current Opinion in Genetics & Development*, vol. 48, pp. 1–7, 2018.

[40] Z. Yang, J. Li, G. Feng et al., “MicroRNA-145 modulates N6-methyladenosine levels by targetting the 3′-untranslated mRNA region of the N6-methyladenosine binding YTH domain family 2 protein,” *Journal of Biological Chemistry*, vol. 292, no. 9, pp. 3614–3623, 2017.

[41] M. Bartosovic, H. C. Molasere, P. Gregorova, D. Hrossova, G. Kudla, and S. Vanacova, “N6-methyladenosine demethylase FTO targets pre-mRNAs and regulates alternative splicing and 3′-end processing,” *Nucleic Acids Research*, vol. 45, no. 19, pp. 11356–11370, 2017.

[42] J. Wen, R. Lv, H. Ma et al., “Zc3h13 regulates nuclear RNA m6A methylation and mouse embryonic stem cell self-renewal,” *Molecular Cell*, vol. 69, no. 6, pp. 1028–1038, 2018.

[43] C. R. Alarcón, H. Lee, H. Goodarzi, N. Halberg, and S. F. Tavazoie, “N6-methyladenosine marks primary microRNAs for processing,” *Nature*, vol. 519, no. 7544, pp. 482–485, 2015.

[44] N. Liu, K. I. Zhou, M. Parisien, Q. Dai, L. Diatckenho, and T. Pan, “N 6-methyladenosine alters RNA structure to regulate binding of a low-complexity protein,” *Nucleic Acids Research*, vol. 45, no. 10, pp. 6051–6063, 2017.

[45] J. Choc, S. Lin, W. Zhang et al., “mRNA circularization by METTL3 elf3h enhances translation and promotes oncogenesis,” *Nature*, vol. 561, no. 7724, pp. 556–560, 2018.

[46] S. Lin, J. Choc, P. Du, R. Triboulet, and R. J. Gregory, “The m 6 A methyltransferase METTL3 promotes translation in human cancer cells,” *Molecular Cell*, vol. 62, no. 3, pp. 335–345, 2016.

[47] W. Wei, B. Huo, and X. Shi, “miR-600 inhibits lung cancer via downregulating the expression of METTL3,” *Cancer Management and Research*, vol. 11, pp. 1177–1187, 2019.

[48] F. Luongo, F. Colonna, F. Calapà, S. Vitale, M. E. Fiori, and R. De Maria, “PTEN tumor-suppressor: the dam of stemness in cancer,” *Cancers*, vol. 11, no. 8, 2019.

[49] A. Gkountakos, G. Sartori, I. Falcone et al., “PTEN in lung cancer: dealing with the problem, building on new knowledge and turning the game around,” *Cancers*, vol. 11, no. 8, 2019.

[50] J. Gu, W. Ou, L. Huang et al., “PTEN expression is associated with the outcome of lung cancer: evidence from a meta-analysis,” *Minerva Medica*, vol. 107, no. 5, pp. 342–351, 2016.

[51] J. Yan, X. Huang, X. Zhang et al., “LncRNA LINCO0470 promotes the degradation of PTEN mRNA to facilitate malignant behavior in gastric cancer cells,” *Biophysical Research Communications*, vol. 521, no. 4, pp. 887–893, 2020.