CHAPTER

10

An Insight of Polyphenols in Lung Cancer Chemoprevention

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Abbreviations

ABCG2	ATP-binding cassette sub-family G member 2
AMPK	adenosine monophosphate protein kinase
AP-1	activator protein 1
B(a)P	benzo[*a*]pyrene
Bad	Bcl-2-associated death promoter
Bak	Bcl-2 homologous antagonist/killer
Bax	BCL2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra-large
C-met	tyrosine-protein kinase Met
CXCL12	C-X-C motif chemokine 12
EF1A	elongation factor 1a
EGCG	epigallocatechin gallate
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
HDAC	histone deacetylase
HER-2	human epidermal growth factor receptor-2
HGFR	hepatocyte growth factor receptor
HIF-1α	hypoxia inducible factor-1 alpha
IALT	international adjuvant lung cancer trial
IKK1	Iκb kinase1
Inos	inducible nitric oxide synthase
JNK	C-Jun N-terminal kinases
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinases
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
Nox5	NADPH oxidase
NSCLC	nonsmall cell lung cancer
PI3K	phosphoinositide 3-kinase
PTEN	phosphatase and tensin homolog
PUMA	P53 upregulated modulator of apoptosis
SA-β-gal	senescence-associated β-galactosidase
SCID	severe combined immunodeficient
SCLC	small cell lung cancer
SIRT1	silent mating type information regulation 2 homolog 1
Sma/Mad	mothers against decapentaplegic
SOD1	superoxide dismutase 1
STAT	signal transducer and activator of transcription
TGFb1	transforming growth factor beta 1
TNFβ	tumor necrosis factor beta
TRAIL	TNF-related apoptosis-inducing ligand
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
WHO	World Health Organisation
XIAP	X-linked inhibitor of apoptosis
ZEB1 gene	zinc finger e-box binding homeobox 1

1 INTRODUCTION

Cancer is a leading cause of death worldwide, accounting for 8.8 million deaths in 2015 according to a World Health Organization (WHO) fact sheet. Lung cancer is a malignant lung tumor characterized by uncontrolled cell growth in lung tissue. Lung cancer accounts for the highest incidence and mortality, 1.69 million deaths, compared to other cancers such as prostate, colon, and breast cancers [1]. Among women, it is the second highest in mortality and third highest in incidence after breast cancer. The reason for the high mortality rate could be the detection of lung cancer at an advanced stage, which is not amenable to surgical or radiation therapy. The poor prognosis of lung cancer is because of the delayed diagnosis and patient mortality is largely attributed to the spread of the metastatic form of cancer within the lung and to distant organs. Major forms of lung cancers have an epithelial origin, originating from the epithelium of the

proximal respiratory tract and also of the bronchi. Lung cancer is pathologically classified into two classes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). This clinical classification is based on their propensity to metastasize and their response to existing therapeutic regimens leading to planning of their clinical management. Of significant importance, approximately 75% of lung cancers are NSCLC with a strong causal link with smoking. NSCLC is less responsive to chemotherapy and is less metastatic than SCLC. Tobacco smoking is the major cause, with 90% of cases attributed to it [2]. In addition to cigarette smoke, which contains carcinogenic nitrosamine derivatives, genetic vulnerability and exposure to different environmental carcinogens encompassing predominantly polycyclic aromatic hydrocarbons serve as major risk contributors to lung carcinogenesis [3,4]. NSCLC is clinically stratified into three primary stages, explicitly: (1) local (IA, IB, IIA), (2) locally advanced (IIB, IIIA, IIIB), and (3) the advanced (IIIB, IV) stages. These stages are further classified depending on size of tumor, status of lymph nodes, and tumor metastases. For NSCLC patients identified with early stages involving stage I to stage IIIA, surgery is the most efficacious option for therapy. Postsurgery, the five-year survival rate of NSCLC patients ranges from <5% for Stage III–IV, 23% for Stage IIIA, to 67% for Stage IA. Patients undergoing resection of lung cancer are associated with a high relapse risk, and the results of the four randomized trials, including those from the National Cancer Institute of Canada intergroup study, the Adjuvant Navelbine International Trialist Association Trial, the International Adjuvant Lung Cancer Trial, and the Cancer and Leukemia group B trial, have reinforced the usage of adjuvant chemotherapy post complete resection of early stages ranging from IIA–IIIA. The combination of chemotherapy with radiotherapy has also shown survival benefits in patients with impossible-to-operate locally advanced and disseminated forms of NSCLC. Prior to 1990, a very few drugs, comprising alkylating agents like cisplatin and ifosfamide, DNA crosslinking agents like mitomycin C, mitotic inhibitors like vinblastine and vindesine, and topoisomerase inhibitors like etoposide were employed against NSCLC. Among these platinum-based alkylating agents, specifically cisplatin was the most effective. Thus in 1995 NSCLC patients in advanced stages, when treated with platinum-based chemotherapy, exhibited an upsurge in median survival of 1.5 months. Following this, new chemotherapeutic drugs like gemcitabine, paclitaxel, and docetaxel were effectively employed in NSCLC patients. To achieve better outcomes with the therapeutic regimen, these drugs have been synergistically used with either carboplatin or cisplatin, specifically, in the treatment of NSCLC patients in advanced stages.

However, it is established from therapeutic outcomes that significant toxicity accompanies platinum-based chemotherapy. Hence, nonplatinum therapeutic regimens like taxane-based treatments have also been explored in NSCLC cases. Additionally, numerous other targeted regimens are being investigated for lung cancer treatment in clinical set-ups, including (i) PI3K/Akt/mTOR inhibitors, for example, rapamycin; (ii) proteasome inhibitors, for example, Bortezomib; (iii) EGFR inhibitors, for example, Gefitinib, Erlotinib; (iv) VEGF/VEGFR inhibitors, for example, Bevacizumab, Vandetanib; (v) HDAC inhibitors, for example, Vorinostat. In conclusion, the systemic usage chemotherapeutic drugs and molecular targeted therapies in the treatment of locally advanced or metastatic NSCLC patients is restricted due to the acquisition of drug resistance and accompanied acute and cumulative dose limiting toxicities. Therefore there is need for developing additional effective approaches. Therapeutic intervention and prevention by phytochemicals, which are cost-effective, nontoxic, and physiologically bioavailable, are evolving strategies in the management of cancer. The numerous benefits accompanying these phytochemicals, over the conventional treatment regimens like radiation therapy and chemotherapy, portray these agents as possible idyllic contenders in chemoprevention approaches [5].

2 CHEMOPREVENTION

Chemoprevention is a credible secondary prevention approach using agents that can interfere with the initiation, promotion, and progression of cancer, targeting both high-risk former and current smokers. Chemoprevention using dietary phytochemicals to impede the progress of the carcinogenesis process could be an effective strategy to augment the risk of lung cancer development [6]. Many research outcomes strongly substantiate the link between a lower cancer risk and a high intake of fruits and vegetables abundant in polyphenols. Extensive research from the past decade has featured polyphenols as potential candidates for anticancer effects and chemoprevention. They are defined as compounds possessing at least one aromatic ring with one or more hydroxyl functional groups attached [7]. They showcase their presence in foods and beverages of plant origins [8]. Polyphenols from natural sources can be chemical classes comprising flavonoids, phenolic acids, lignans, and stilbenes. The most common classes are constituted of flavonoids and phenolic acids, which account for 60% and 30% of the entire polyphenol class [9]. Abundant scientific studies have proven that anticancer efficacy of natural polyphenols could be an outcome of their potent antioxidant and antiinflammatory activities and their ability to modulate molecular targets and signaling pathways involved in cell survival, proliferation, migration, differentiation, angiogenesis, detoxification enzymes, hormone activities, immune responses, etc. [10,11]. Therefore, in

addition to cessation of smoking, dietary intake of polyphenols could serve as a striking methodology for the control and prevention of lung cancer. In this chapter we discussed the use of selected dietary phytochemicals, including green tea polyphenols, pomegranate polyphenols, silibinin, genistein, curcumin, resveratrol, fisetin, quercetin, and luteolin as chemopreventive and/or chemotherapeutic agents for the prevention and treatment of lung cancer.

3 POLYPHENOLS AND LUNG CANCER

3.1 Green Tea Polyphenols

Tea, obtained from the plant *Camellia sinensis*, is one of the common beverages consumed worldwide. Numerous scientific studies from the literature have proven the cancer-preventive action of tea consumption [12]. (−)-Epigallocatechin gallate (EGCG) is the major constituent of green tea contributing to its beneficial effects. Preventive or therapeutic activities of green tea and its components, explicitly EGCG, are attributed to a plethora of mechanisms, including modulation of enzymes responsible for metabolism of carcinogens and cell signaling pathways, stimulation of apoptosis arrest of cell cycle, and inhibition of stimulation of transcription factors leading to suppression of development and progression of cancer. An in vitro study using human lung cancer cells revealed that EGCG treatment obstructed their anchorage-independent growth mediated through up-regulation of p53 expression, augmented p53 phosphorylation and transcription, and suppressed p53 ubiquitination mediated through mouse double minute 2, a negative regulator of the p53 tumor suppressor. Thus the study proposed P53 as a potential target for lung cancer prevention exhibited by EGCG [13]. Further exposure of NSCLC cells to EGCG in the concentration range 5–20 μM was found to inhibit transforming growth factor-induced epithelial-mesenchymal transition via down-regulation of phosphorylated Sma/Mad related protein and extracellular signal-regulated protein kinases 1 and 2 [14]. Moreover, treatment of resistant as well as sensitive small cell lung carcinoma (SCLC) cells with EGCG was found to cause 50%–60% reduction in telomerase activity and decrease in caspases-3 and -9 activities along with fragmentation of cell DNA and arrest of cell cyclin S-phase [15]. When tested in erlotinib sensitive and resistant cell lines with acquired resistance and c-Met growth factor overexpression, EGCG resulted in inhibition of cell proliferation, proposing the therapeutic potential of EGCG therapy in erlotinib-resistant patients [16]. EGCG and green tea polyphenols exhibited preventive and therapeutic effects in in vivo lung cancer models. Dietary ingestion of EGCG as 0.1%, 0.3%, and 0.5% in the diet resulted in inhibition of tumor growth in H1299 cells in implanted athymic nude mice. The mechanism for growth inhibition was shown to involve apoptosis and oxidative DNA damage through induction of reactive oxygen species generation [17]. Further preclinical studies using Swiss albino mice indicated that administration of green and black tea polyphenols at doses of 0.1% and 0.2% resulted in reduced occurrence of alveologenic tumors induced by diethylnitrosoamine in lungs of animal models mediated through suppression of the expression of Akt, cyclooxygenase and nuclear factor kappa-B [18]. Further, a study involving lung cancer cells from human and mouse origin revealed that EGCG up-regulates expression of miR-210, a major miRNA regulated by HIF-1α, thereby reducing the proliferation rate of cancerous cells and their anchorage-independent growth [19]. It has been established that EGCG and theaflavin could decrease the proliferative index at diverse stages of experimental lung carcinogenesis in a benzo (a) pyrene-induced lung carcinogenesis mouse model. Dosing of theaflavins at an amount of 0.02 mg/mouse/day and EGCG at an amount of 0.01 mg/mouse/day was found to reduce in-situ dysplasia, hyperplasia and carcinoma being apparent on 8th, 17th, and 26th weeks respectively [20]. Treatment of Swiss albino mice with green tea polyphenols and black tea polyphenols at 0.1% and 0.2% doses resulted in reduced occurrence of diethylnitrosoamine induced alveolar tumors. In addition, it led to a down-surge of expression of Akt, cyclooxygenase-2, and NF-κB [18]. Combination of 0.25% or 0.5% of polyphenon E in drinking fluid and atorvastatin resulted in inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone induced lung tumorigenesis in a mouse model. This low-dose combination of atorvastatin and polyphenon E resulted in significant decline of lung tumor burden and tumor multiplicity with augmentation of apoptosis and inhibition of myeloid cell leukemia 1 level in adenomas. Thus the study proved that the atorvastatin and polyphenon E combination significantly suppressed lung tumorigenesis. In both in vitro and in vivo studies, the effect of the two drugs was found to be synergistic [21]. Dietary ingestion of polyphenon E at a concentration of 1% wt/wt and exposure to difluoromethylornithine aerosol was explored in A/J mice injected with B(a)P. Administration of polyphenon E did not result in inhibition of average tumor multiplicity but led to decreased tumor load per animal with a substantial reduction in largest carcinomas [22]. Further ingestion of Polyphenon E at a concentration of 0.5% in drinking fluid with caffeine was found to cause substantial reduction in the number of visible lung tumors and occurrence and growth of lung adenocarcinoma induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mice. This effect was associated with reduction of cell proliferation, apoptosis induction, and reduced levels of p-extracellular signal-regulated kinase (ERK) 1/2 and c-Jun in tumor cells, which might contribute to the

progression/inhibition of lung adenoma to adenocarcinoma [6]. Moreover, green tea solution at concentrations of 0.1%, 0.2%, 0.4%, and 0.6% was administered to female A/J mice induced with lung tumorigenesis using 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. It was observed that only 0.6% green tea solution treatment leads to reduction in multiplicity of lung tumors in mice and resulted in suppression of angiogenesis and augmentation of apoptosis in lung adenomas [23]. In the following study, oral administration of green tea polyphenols at a concentration of 5 mg/0.2 mL, prior to the administration of the carcinogen, resulted in protection against lung tumorigenesis [24].

3.2 Pomegranate Polyphenols

Pomegranate, botanically known as *Punica granatum*, is an edible fruit enriched with therapeutically important phytochemicals, including punicalagin and ellagitannins, predominantly present in the fruit peel. Treatment of human lung cancer A549 cells with pomegranate fruit extract, at concentrations of 50–150 ppm, led to cytotoxic effects with arrest of cell cycle in the G_0–G_1 phase, reduced expression of cyclins and cyclin-dependent kinases, and enhanced cell cycle regulatory proteins. Further, it was found to suppress PI3K, MAPK, Akt, and NF-κB phosphorylation. In addition, no toxic effects were seen on normal bronchial epithelial cells. Athymic nude mice induced with tumor, using human lung cancer A549 cells, following oral intake of pomegranate fruit extract showed significant prolongation of the tumor latency period and reduction of the tumor growth leading to enhanced survival rate [25]. Further, pomegranate fruit extract when orally administered at a dose of 0.2%, *w*/*v* in two A/J mouse models, with lung cancer induced using benzopyrene and N-nitroso-tris-chloroethylurea, caused a significant reduction in tumor spreading and incidences. In addition, it blocked PI3K, NF-κB, MAPK, and phosphorylation of mTOR, Akt, c-met, markers of angiogenesis and cell proliferation. These results substantiate the therapeutic potential of pomegranate fruit extract in the chemoprevention of lung cancer patients. Further, this protective effect was attributed to modulation of signaling pathways and related events, critical for the formation and progression of lung cancer [26].

Ellagitannins at a concentration of 50–500 μM and punicalagin at a concentration of 50–500 μM when investigated were found to cause a noteworthy suppression of the resultant DNA adducts on incubation of B(a)P with rat liver microsomes, suitable cofactors, and DNA [27]. In another in vitro study, administration of pomegranate peel aqueous extract was found to inhibit phorbol myristate acetate-stimulated neutrophils and luminol-amplified chemiluminescence of resting neutrophils dose dependently, resulting in direct in-vitro inhibition of myeloperoxidase activity [28].

3.3 Silybinin

Silymarin, obtained from milk thistle plant, botanically known as *Silybum marianum*, is widely used for its hepatoprotective effect against different liver conditions. The major constituent of the silymarin complex is known as silybinin. Several studies have reported inhibition of tumor proliferation by silybinin in a variety of cancers, ranging from prostrate, ovarian, skin, breast, and bladder to lung cancer [29]. The chemoprevention activity of silybinin in lung cancer has been comprehensively explored in in vivo and in vitro systems. Studies have proven that silybinin effectively inhibits the growth of lung cancer cells when used alone and in combination with chemotherapeutic drugs. In athymic BALB/c nu/nu mice, silybinin inhibited the A549 xenograft growth in human non-small-cell lung carcinoma [30]. In a urethane-induced lung cancer A/J mouse model, oral intake of silybinin was found to inhibit tumorigenesis and decrease tumor size by altering proteins involved in cell proliferation, angiogenesis, and inflammation. Moreover, in in vivo silybinin was found to inhibit VEGF secretion and microvessel lung tumors [31]. Further, silybinin was observed to inhibit invasion and motility of A459 lung cancer cells by down-regulating expression of tissue activators like matrix metalloproteinase-2 and urokinase-type plasminogen activator via decreased phosphorylation of Akt and ERK1/2, and up-regulating expression of tissue inhibitors like metallopeptidase-2. [32,33]. In another in vitro study, silybinin in the concentration range of 25–100 μM exhibited cytotoxic activity in SHP-77 small cell lung carcinoma (SCLC) and A549 non-small-cell lung carcinoma (NSCLC) cells [34]. In addition to inhibition of cell growth, silybinin induced apoptosis in a dose-dependent manner and caused alterations in cell cycle regulation. Follow-up studies in different NSCLC cell lines, including H1299, H460 and H322, also revealed cytotoxic activity of silybinin [35]. Moreover, treatment with silybinin was observed to suppress lung metastasis in an HER-2/neu transgenic mouse model [36]. Further studies showed that silybinin suppresses primary growth of lung tumor and its progression in mice, in addition to down-regulation of inducible nitric oxide (iNOS) expression in the treatment group. iNOS levels are positively correlated with the metastatic and angiogenic potential of tumors [37]. A study involving tumor-derived LM2 mouse lung epithelial cells reported modulation of signaling cascades regulating COX-2 and iNOS by silybinin. Following treatment with silybinin, a reduction in cytokine induced activation of STAT1, STAT3, and ERK1/2 and NF-κB-DNA binding [38,39].

Moreover, the studies exploring its metastatic potential showed that silybinin targets the epithelial to mesenchymal transition by enhancing levels of epithelial markers and reducing the levels of mesenchymal markers [40,41]. Further study demonstrated that silybinin, on coadministration with erlotinib for 24 days, led to induction of apoptosis and irreversible inhibition of tumor growth in PC-9 erlotinib-resistant tumors in SCID mice. In addition, the study revealed decreased Akt and EGFR activity and uptake of 18F-fludeoxyglucose in tumors [42]. Silybinin was also found to potentiate the cytotoxic effect of indole-3-carbinol against nicotine-derived nitrosamine ketone-induced lung adenocarcinoma in A/J mice [43]. Further exploration of epigenetic studies demonstrated that silybinin reduces histone deacetylase HDAC [1–3] protein levels and leads to build-up of acetylated histones (H3 and H4) in chromatin. The outcomes strongly suggested the use of silybinin in combination with histone deacetylase inhibitors to improve their activity, to obtain better clinical efficacy in NSCLC patients [44]. E-cadherin expression is one of the widely used clinical biomarkers for prediction of responses to conventional lung cancer therapies [45]. In another epigenetic study, silybinin was observed to reduce the Zeb1 levels, the main transcriptional repressor of E-cadherin, and also to increase expression of the E-cadherin protein in NSCLC cells, thus highlighting the significance of silybinin in down-regulation of EMT-related events by silybinin [46].

3.4 Genistein

Genistein, abundantly found in soybeans, is chemically an isoflavone. It is widely reported for its chemotherapeutic and chemopreventive effects and has been demonstrated to show its antitumor effects through several pathways including triggering of apoptosis, induction of cell cycle arrest, and inactivation of perilous signaling pathways in human cancer cells. Genistein when tested in H446 cells was found to significantly suppress the proliferation and migration ability of these cells, associated with G2/M phase cell cycle arrest and apoptosis. Moreover, genistein was found to potentiate the antiproliferative effect of cisplatin on these cells. To highlight study outcomes, genistein caused inhibition of the forkhead box M1 protein, a major contributor of DNA damage responses and down-regulated an array of FoxM1 target genes controlling cell cycle and apoptosis [47]. When explored in human lung carcinoma A549 cells, genistein potentiated trichostatin A-induced apoptosis mediated by upsurge of caspase-3 activity and tumor necrosis factor receptor-1 signaling [48,49]. In the following study, genistein treatment led to augmentation of the antitumor effects of epidermal growth factor receptor inhibitors and tyrosine kinase inhibitors, in different subtypes of lung cancer cell lines, including H3255, H1650, and H1781 NSCLC cell lines. The cytotoxic effect was accompanied by reduction in the expression of COX-2, EGFR, prostaglandin E2, p-Akt, and down-regulation of DNA-binding activity of NF-κB. Further, genistein treatment in SPC-A-1 human lung adenocarcinoma cell lines inhibited apoptosis induction and proliferation and caused cell-cycle arrest, via regulation of several apoptotic-related genes [50].

When C57BL/6 mice were treated with genistein, it caused mitigation of lung tumor nodules, content of lung collagen hydroxyproline, and serum sialic acid level, leading to enhanced lifespan of C57BL/6 mice [51]. Moreover, when tested in a xenograft mice model, genistein potentiated the anticancer effect of gefitinib [52].

3.5 Curcumin

Curcumin is a polyphenol derived from the plant *Curcuma longa*, which has been widely explored for its antiangiogenic, antioxidant, analgesic, antiinflammatory, and antiseptic properties [53]. Exposure of NSCLC cells to curcumin alleviated the invasion and proliferation of these cells, and surged arrest of the G_0/G_1 phase cell-cycle through blockade of metastasis-associated protein 1 regulated inhibition of the Wnt/β-catenin pathway [54]. Treatment of human lung adenocarcinoma cells with curcumin resulted in induction of autophagy mediated through stimulation of the AMPK signaling pathway and up-regulation of acetylCoA carboxylase (AMPKα) and AMP-activated protein kinase [55]. Further, curcumin was found to target transcription (STAT)-3 signaling in the SCLC progression leading to tumor suppression [56]. In preclinical studies on administration to C57BL/6 mice, curcumin led to inhibition of the primary tumor growth of Lewis lung carcinoma. In cancerous tissues, curcumin exhibited α1-antitrypsin up-regulation and reduced neutrophil elastase levels, substantiating the role of curcumin in modulation of lung tumor proliferation in inflammation-associated microenvironments [57]. In a further exploration, inclusion of 1% curcumin in the diet inhibited K-ras-initiated lung cancer and chronic obstructive pulmonary disease, such as airway inflammation in mice [58]. Recently, mechanistic studies demonstrated that the proapoptotic effects of curcumin in non-small cell lung cancer rely on induction of miR-192-5p/215 and the p53-miR-192-5p/215-XIAP pathways [59].

Curcumin suppressed tumor sphere growth of NCI-H460 lung cancer cells in vitro and in vivo [60]. Zhu et al. further reported that curcumin exhibits its effect on lung cancer stem cells through inhibition of Sonic Hedgehog and Wnt/β-catenin pathways [61]. Moreover,

administration of curcumin in the diet to a benzo[*a*]pyrene-induced carcinogenesis Swiss albino mouse model resulted in inhibition of MAPK and NF-κB signaling and Cox-2 transcription in lung tissues of mice [62]. Thus findings from several studies have strongly substantiated the therapeutic potential of curcumin in lung cancer elimination and cancer intervention.

3.6 Resveratrol

Resveratrol is a natural polyphenol, abundantly occurring in grapes, peanuts, and red wine. The root of *Polygonum cuspidatum*, extensively used in traditional Japanese and Chinese medicine, is a rich source of resveratrol. Grape skin contains 0.5%–1% resveratrol [63]. Resveratrol has been widely investigated as a standalone treatment and in combination with other drugs in several lung cancer cells. Treatment of A549 and H460 cells with resveratrol was found to suppress growth, accompanied by up-regulation of microtubule-associated protein 1 light chain 3 and enhanced accumulation of proline-, glutamic acid-, and leucine-rich protein-1 in autophagosomes [64]. Further treatment of A549 and H1299 cells with resveratrol exhibited a dose-dependent growth inhibition, in an apoptosis-independent mechanism. The probable mechanisms for this effect were proposed to be upsurge of p53, p21SA-β-gal, Nox5 expression, double-stranded DNA breaks, ROS levels, and declined EF1A expression [65]. Another study treatment of A549 cells with resveratrol after exposure to Benzo(a)pyrene led to alleviation of cell viability, down-regulation of Bcl-2, NFkB and Ikk1 expression, arrest of G2/M cell cycle, elevation of p53 levels, and an induction of apoptosis, reduced cyclin D expression, and enhanced p21 and TRAIL receptors 1 and 2 expression [65]. Moreover, when explored for antimetastatic activity in A549 cells, resveratrol treatment resulted in decreased cell proliferation, blockade of TGFβ1 induced epithelial to mesenchymal transition, and inhibition of cell adhesion [66]. Further, treatment of H322, CL-1-5, A549 and H1435 cells with resveratrol resulted in suppression of growth and down-regulation of Akt, IκB, and NF-κB. Exposure of H727 cells to cigarette smoke condensate following treatment with resveratrol conjugated nanoparticles revealed the potential of resveratrol to attenuate cigarette smoke condensate-induced DNA fragmentation in these cells [67]. Several animal studies evaluating anticancer activity of resveratrol in lung cancer animal models substantiated the efficacy of resveratrol. Nude mice induced with subcutaneous injection of A549 cells on treatment with 15, 30 or 60 mg/kg resveratrol for 15 days exhibited inhibition of lung tumor growth in a dose-dependent fashion [68]. Further, C57BL/6 mice injected with Lewis lung carcinoma cells, when treated with resveratrol, showed inhibition of lung tumor growth mediated through decrease of M2 polarization and decreased F4/80+ expression [69].

Several studies in the literature have reported findings from investigations of resveratrol as a part of combination treatment. Pretreatment of A549 and H460 cells with resveratrol followed by IR exposure resulted in a synergistic effect in NSCLC. The synergism could be attributed to an apoptosis-independent mechanism which, in turn, was observed to enhance the percentage of SA-β-gal positive senescent cells and also the double-stranded DNA breaks [50]. Treatment of H-2452 cells with resveratrol in combination with Clofarabine resulted in a synergistic alleviation in expression of Msl-1 protein with insignificant effect on expression of Bcl-xL. Nevertheless, it was observed that knockout of Bcl-xL protein resulted in augmentation of the ability of combination treatment to suppress cell proliferation and enhance apoptosis [70]. The enhancement in apoptosis observed through the treatment of the cancer cells was shown to be facilitated through arrest of the G2/M phase cell cycle and enhanced caspase-7 and -3 activity and cleavage of caspase-3 [71]. Further, treatment of diverse cell lines including H1975, H460, A549, and PC-9 with an Erlotinib and resveratrol combination led to a significant increase of apoptosis induction by Erlotinib. In addition, it caused decreased cell viability and colony formation [72]. A rise in generation of ROS, caspase-3 activity, and decrease in expression of antiapoptotic proteins including Mcl-1 and surviving and expression of PUMA and p53 promotion was observed. In addition, the combination was found to inhibit the Akt/mTOR/p70s6K pathway [73]. In the following study, A549 cells were cultured as spheroid bodies to mimic cancer stem cells. These cancer stem cells were transfected with ZD55 oncolytic adenovirus harboring the TRAIL gene, followed by treatment with resveratrol. Resveratrol was found to augment the ZD55-TRAIL mediated cytotoxicity compared to stand-alone treatment with ZD55-TRAIL [74]. Further, it was observed that apoptosis induction by these cells was associated with caspase dependency accompanied by reduced levels of pro-caspase-8, -9 and -3. Further treatment of PC9 cells with a Gefitinib and resveratrol combination led to decreased synergistic suppression of growth of Gefitinib-resistant NSCLC cell lines. In addition, this cotreatment resulted in apoptosis induction, arrest of cell cycle, autophagy, and senescence [75]. These beneficial effects were found to be routed through suppression of EGFR phosphorylation by enhancing intracellular accumulation of Gefitinib, enhanced expression of CYP1A1 and ABCG2, cleavage of LC3B-II, caspase-3, p53 and p21 [52]. In another study, pretreatment of lung fibroblasts affected with idiopathic pulmonary fibrosis with resveratrol followed by CXCL12or TGF-β revealed that resveratrol represses conversion of fibroblast to

myofibroblast in lung cells. Further, it was observed that resveratrol has the potential to cause reversal of fibroblast to myofibroblast [76]. In a preclinical study 5-week-old female nude mice subcutaneously injected with A549 cells and intraperitoneally treated with Dehydrosilybin, a resveratrol analog at a dose of 50 mg/kg, showed reduced tumor growth [23]. Further, nude mice injected with A549 cells on alternate treatment with 20 mg/kg of resveratrol for 25 days resulted in suppression of metastasis and SIRT1 activation [51]. Moreover, male mice were exposed to 100 mg/kg dose of Benzo(a)pyrene in order to induce carcinogenesis in lungs. Following this, the mice were treated with resveratrol at a concentration of 5.7 μg/mL suspended in drinking water in combination with 60 mg/kg body weight of curcumin for 22 weeks. Mice treated with benzopyrene alone showed enhanced p53 hyperphosphorylation and reduced caspase-9 and -3 activity. The cotreatment resulted in an upsurge in caspase-3 and -9 activity and downsurge of p53 hyperphosphorylation, thus alleviating the effects of the benzopyrene treatment [53].

3.7 Fisetin

Fisetin, chemically known as 3,3′,4′,7-tetrahydroxyflavone, is one of the naturally occurring flavonoids with occurrence in strawberry, cucumber, grape, persimmon, apple, and onion. It is reported to possess apoptotic, antiproliferative, and antiagiogenic properties in cancer research [77]. A critical hurdle for chemotherapy in non-small cell (NSC) lung cancer is the resistance developed to the commonly employed chemotherapeutic drug cisplatin. Fisetin, when tested in A549-CR lung cancer cells, was found to cause reversal of the acquired resistance to cisplatin. In addition, fisetin when combined with cisplatin exhibited an immense downsurge of cell viability and apoptosis induction compared to standalone treatments with fisetin and cisplatin, the effect mainly attributed to inactivation of MAPK pathways and reduced expression of survivin [78]. During exploration of the effect of fisetin in NSCLCs, it was shown to induce apoptosis mediated through mitochondrial pathways. In addition in NCI-H460 cells, fisetin was shown to induce ROS generation, DNA fragmentation, caspase-3 activation, and apoptosis through Bcl-2 decrease and enhanced expression of Bax [79]. A recently published study further proved the apoptosis-triggering potential of fisetin through endoplasmic reticulum stress mediated apoptosis in NCI-H460 cells [80]. Fisetin was investigated in combination with paclitaxel in A549 cells. In the results it was found to evidence arrest of the G2/M cycle, inhibition of mitosis, distortion of the chromosome, suppression of cytokinesis, and more prominently induction of autophagic cell death [81]. In the following study, fisetin exerted an anticancer effect against HCC827-ER, erlotinib-resistant lung adenocarcinoma cells [82]. In a study using A549 NSC lung cancer cells, outcomes revealed that fisetin activates PTEN, a tumor suppressor, and down-regulates protein synthesis via AMPK phosphorylation. In addition, it was also found to inhibit the PI3K/Akt/mTOR signaling pathway [83]. Following study revealed that fisetin suppressed migration, adhesion, and invasion in A549 lung cancer cells by negatively regulating ERK1/2, uPA and MMP-2. Further, it reduces the nuclear levels of NF-κB, c-Fos, AP-1 c-Jun, and blocks binding of NF-κB [84]. In preclinical studies, administration of fisetin to an (a) pyrene (B(a)P)-induced lung cancer animal produced decreased dysfunction of mitochondria and induction of apoptosis via Bax/Bcl-2 ratio upregulation, thus resulting in release of cytochrome-c and caspase-9, caspase-3 activation, ultimately leading to apoptotic cell death [85]. Overall, numerous in vitro and in vivo studies have substantiated the potential of fisetin for treatment of lung cancer.

3.8 Quercetin

Quercetin, chemically known as 3,3,4,5,7-pentahydroxyflavone, belongs to the flavonol subgroup of flavonoids and is one of the most extensively explored polyphenols. It is well documented in research outcomes that ingestion of quercetin in higher amounts is potentially and negatively correlated with lung cancer risk. Quercetin is reported to exhibit inhibition of growth in cancer cell lines. Prophylactic cotreatment of quercetin and curcumin in lung cancer resulted in apoptosis induction mediated via p53 posttranslational modulation [86]. Proposed mechanisms for inhibition of proliferation by quercetin in cancer cells include suppression of activity of cyclin-dependent kinases, reduced cyclin levels, and inhibition of cell survival pathways including the PI3K/Akt/NFkB pathway. Further, quercetin promoted apoptotic cell death as an outcome of several events, including activation of stress proteins, the generation of reactive oxygen species, the suppression of antiapoptotic proteins, and activation of proapoptoic B-cell lymphoma 2 members and of the caspase cascade. The mechanism of action of quercetin has been widely explored in numerous cancer cell lines. To explore the mechanism in NSCLC, the influence of quercetin on the cytoskeleton of lung cancer cells has been investigated. Cytoskeletal proteins are involved in the regulation of major processes implicated in tumorigenesis, like cell migration, proliferation, and cell death. Quercetin, when tested in metastatic A549 cells, was found to change the arrangement of major cytoskeletal proteins, F-actin, -tubulin and vimentin [87]. Quercetin, when tested in non-small cell lung cancer cell lines, was found to inhibit migration or invasion. Further,

it was found to inhibit bone metastasis in an orthotopic A549 xenograft model mediated by inhibition of epithelial-to-mesenchymal transition. In addition, animal survival time was found to be prolonged after treatment with quercetin. In mechanistic explorations, quercetin was found to inhibit Snail-dependent activation of Akt by enhancing maspin. In addition, quercetin modulated the invasive ability of NSCLC cells by up-regulating expression of Snail-independent disintegrin and metalloproteinase 9 pathways [88]. In the following study involving A549 cells, treatment with quercetin was found to trigger BCL2/BAX-mediated apoptosis, necrosis, and mitotic catastrophe and inhibit migratory potential. Reduction in migration of A549 cells following quercetin treatment was attributed to the stripping effect of quercetin on microtubules, microfilaments, and vimentin filaments, in addition to the inhibitory effect of quercetin on expression of vimentin and N-cadherin. Moreover, another possible mechanism involved in mitotic catastrophe induced by quercetin was the disconcertion of mitotic microtubules resulting in monopolar spindle formation, and subsequently leading to the cytokinesis failure. Quercetin was also proposed to deplete actin filaments contributing to cytokinesis failure [89].

3.9 Luteolin

Luteolin is a naturally occurring flavonoid found as one of the constituents of flowering plants, in particular food plants. Luteolin is known to induce accumulation of O_2 while it reduces the H_2O_2 concentration in lung cancer cells. Conversion of O_2 to H_2O_2 happens through the manganese superoxide dismutase (MnSOD) enzyme and suppression of MnSOD activity was observed with luteolin, but it remains to be determined whether some other mechanisms lie beneath luteolin-induced peroxidation. ROS interference in the cellular signaling might also contribute to luteolin-induced cancer cell apoptosis. It has been found that luteolin-induced oxidative stress causes suppression of the NF-κB pathway while it triggers JNK activation, which accentuates TNF-induced cytotoxicity in lung cancer cells [90]. It was demonstrated that the antioxidant activity of luteolin is integrated with apoptosis in lung cancer cell line CH27. However, the activation of SOD-1 and SOD-2 proteins by luteolin is modest, and no causative relationship has been established between the induction of SOD proteins and suppression of ROS or apoptosis. in vivo experiments in nude mice with xenografted tumors showed luteolin suppressed growth of tumors formed from mouse Lewis lung carcinoma in a dosage-dependent manner, which is in agreement with in vitro results [91]. Luteolin induces apoptotic cell death via both potentiation of apoptosis pathways and suppression of cell survival pathways. Also, luteolin activates the intrinsic apoptosis pathway by activating p53 and inducing DNA damage [92], and this happens by inhibiting DNA topoisomerase enzymes [93]. Further, Luteolin triggers sustained JNK activation that can promote the apoptosis pathway, presumably through modulation of p53 or BAD. Apoptosis is facilitated by the JNK mediated p53 activation, which results in transcriptional expression of Bax [94]. JNK activation causes mitochondrial translocation of Bax and Bak, which initiate the intrinsic pathway of apoptosis [95]. Luteolin suppresses cell survival pathways to decrease the threshold of apoptosis, on the other hand. As already mentioned, luteolin inhibits a number of survival pathways, such as PI3K/Akt, NF-κB, and MAPKs in cancer cells, which may mimic deprivation of growth factors that block the growth factor-triggered signaling pathways. By suppressing the death receptors-mediated cell survival pathway, NF-κB elevates apoptosis induced by the cognate ligands such as TRAIL or TNFβ. It has been known that TNFβ plays a crucial role in inflammation-associated carcinogenesis via NF-κB-mediated proliferation and cell survival [96]. Luteolin blockage of NF-κB shifts the cell survival and death balance towards the side of death [97] by converting TNFβ from a tumor promoter to a tumor suppressor. TRAIL can potentiate proliferation and metastasis in TRAIL-resistant cancer cells through a mechanism involving NF-κB [98]. Thus, luteolin suppression of NF-κB can sensitize cancer cells to TRAIL-induced apoptosis and prevent the detrimental effect of TRAIL. Luteolin subdues generation and secretion of cytokines such as TNFβ and IL-6, which activate migration and metastasis of cancer cell [99]. TNFβ also activates expression of molecules associated with migration and metastasis of cancer cells, including intercellular adhesion molecule-1. This expression of molecules is inhibited by luteolin [100]. MMP-1 expression is induced by interleukin-6. The generation of IL-6 and MMP-1 expression induced by IL-6 was found to be inhibited by luteolin [101]. Further, luteolin was found to inhibit crucial signal transduction pathways for, for example, EGFR, involved in migration and metastasis of cancer cells [102]. Twist is a transcription factor, critical for epithelial-mesenchymal transition, facilitating metastasis. MMPs are the proteins involved in various stages of metastasis, including the migration of individual cancer cells from the primary tumor, their extravasation, intravasation, and formation of secondary sites of tumor foci [103]. Luteolin was also found to inhibit NF-κB factor crucial for the expression of Twist and MMP. Increased invasive potential could be attributed to focal adhesion kinase (FAK) activity in human carcinoma cells. Luteolin inhibits FAK's cell invasion ability by inhibiting FAK phosphorylation [104]. Additionally, in the following study luteolin was found to inhibit MMP or activity of hyaluronidase enzyme, involved in the maintenance of the neovascularization

barrier. This effect can significantly contribute to suppression of cancer cell metastasis [105]. A number of in vitro studies have revealed that luteolin significantly inhibits migration and invasion of tumor cells through blockade of the PI3K-Akt and MAPK/ERKs pathways [106].

4 CONCLUSION

Despite the development of advanced molecular targeted therapies, lung cancer is the foremost global cause of cancer-related deaths. Moreover, the appalling five-year survival rate for lung cancer patients has not improved during the last several years, demonstrating an urgent need for developing newer therapies that are effective against lung cancer. Cessation of smoking and chemopreventive approaches are two major strategies that can decrease the huge number of lung cancer-associated deaths. Numerous studies reported in the literature have credibly demonstrated the chemopreventive and anticancer potential of polyphenols. The biological significance of polyphenols in lung cancer is attributed to their beneficial properties, which include acceptability, nontoxicity, and safety for human intake. To summarize, significant findings from the literature have highlighted the chemopreventive and anticancer properties of polyphenols in lung cancer. A few polyphenols like silybinin, curcumin, and resveratrol are found to be effective either alone or in combination with other agents, including chemotherapeutic and epigenetic agents, in substantially inhibiting the growth of lung cancer cells. Therefore, on the basis of these studies, the strong chemopreventive potential of polyphenols is well evidenced for their therapeutic usage in clinical set-ups for prevention and treatment of lung cancer.

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