Lung Cancers and the Roles of Natural Compounds as Potential Chemotherapeutic and Chemopreventive Agents

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Among all types of human cancers, lung cancer is one of the most common and has the highest mortality rate. Two major groups of lung cancer based on histological features are non-small cell lung cancers (NSCLCs) and small cell lung cancers (SCLCs). NSCLCs are further subdivided into few subtypes such as adenocarcinoma(AD), squamous cell carcinoma (SCC) and large cell carcinoma(LCC). Focusing on specific treatments or molecular targeted therapy for each type of lung cancers promising the better results as each subtype of lung cancers differ in genomic alterations, the cell of origin and growth pattern. Numerous studies have been done to search for the best chemotherapeutic and chemopreventive agents that can reduce the burden of lung cancer, primarily focusing on potential natural products. This review is focused on genomic alterations and current potential natural compounds as chemotherapeutic and chemoprevention agents specifically on each subtype of lung cancers.

Keywords: Lung cancers; Non-small cell lung cancers; Small cell lung cancer; chemotherapeutic; chemoprevention; natural compounds.

The statistics reported by the World Health Organization (WHO) showed that lung cancer (including trachea and bronchus cancer) is one of the leading cause of deaths worldwide, causing about 1.7 million deaths in 2015[1]. Lung cancer can be due to inborn genetic defects, environmental and lifestyle factors. While inborn genetic defects have little contribution, both environmental and lifestyle factors play a pivotal role in causing lung cancer[2, 3]. Asbestos, ionizing radiation, sulfur mustard, coal-tar pitch and tobacco smoking are some examples of environmental and lifestyle factors that serve as lung carcinogens[4]. Tobacco smoking has been reported as the most influential risk factor for lung cancer cases in 90% of men and 80% of women[5]. According to the International Agency for Research on Cancer, cigarette smoke contains 4000 identifiable chemicals with more than 60 chemicals are carcinogens[6]. Polycyclic aromatic hydrocarbons (PAHs), N-nitrosamine, and aromatic amines are some of the most potent carcinogens that can be found in the cigarette smoke[7]. Based on histological observation under the light microscopy, the two major groups of lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is further

sub-divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma and others[8, 9]. NSCLC is more dominant and makes up almost 85% of all lung cancers while SCLC counts for about 15% of all lung cancers[10]. The most common subtypes of NSCLC are lung adenocarcinoma and lung squamous cell carcinoma (SCC)[11, 12].

The high mortality rate of lung cancer patients is due to the late diagnosis, with 70% of lung cancer cases identified in advanced stages[13]. Late diagnosis has led to the failure of cancer treatment such as chemotherapy and radiotherapy[14]. Early detection of lung cancer can substantially increase the chances of survival, where 70-90% of lung cancer patients with NSCLCs in early stage (stage I) that have been through surgical resection procedure can survive for five years[15, 16]. Difficulty in the detection of an early stage of lung cancer, high cost in diagnostic tests, and invasive diagnostic procedures have been linked to the late diagnosis of lung cancer with most patients were diagnosed with stage IIIB and IV[17]. A population-based study also showed that approximately 75% of lung cancer patients were diagnosed at the advanced stages such as stage III and IV[18]. Tables 1 provides the information about the latest 8th TNM staging system for NSCLCs and the explanation for each stage that differentiate based on three characteristics including the primary tumour, involvement of lymph node and state of metastasis[19].

Treatments

The current treatment for lung cancer includes surgery, adjuvant, chemotherapy, and radiotherapy. The treatment of lung cancer depends on the histology and tumour stage. For example, in NSCLC, surgery will be done in patients with stage I, II, and IIIa if a tumour is resectable and depends on patient's toleration toward surgery[20]. However, patients who have stage IIA, IIB, and IIIA NSCLC need subsequent treatment after the surgical procedure such as chemotherapy[21]. Differently, the first line treatment for SCLC patients is chemotherapy (combination of platinum and etoposide) as surgical resection is rarely done due to early metastasis in this type of lung cancer. SCLC is very sensitive to chemotherapy during the initial treatment, but most of the SCLC patients (up to 90%) tend to develop chemoresistance. Besides, chemotherapy is also the only therapeutic option

for the patients with metastatic NSCLC though most of the patients die as they cannot be treated through surgery procedure[22].

The only curative treatment for lung cancer is a radical surgery[23], yet, delay in diagnosis will lead to the poor prognosis. Unfortunately, 40% of the newly diagnosed NSCLC cases were advanced to stage IV and surgery alone is not enough for the treatment of lung cancer patients in the late stage. Cytotoxic chemotherapy is the first line therapy for NSCLC patients with stage IV that includes four to six cycles of platinum-based therapy such as cisplatin and carboplatin, with maintenance therapy afterwards in some patients is needed[24-26]. There have been many reports on several complications, and side effects of conventional treatment approaches including the development of drug resistance in cancer cells due to the application of synthetic chemotherapeutic agents and the cost for synthetic drugs itself in cancer treatment is more expensive[20, 27]. Furthermore, most of the drugs that used in current cancer treatments have many disadvantages such as rapid metabolism, non-selective, irritating nature, low aqueous stability, and others that consequently lead to the several adverse effects such as low quality of life in cancer patients and dose-limiting side effects [28]. Alternative strategies needed to control this disease and there are many aspects should be improved in lung cancer treatments such as chemotherapy due to insignificant improvement in the efficacy of treatment in recent decades[29].

Chemoprevention

Sporn first introduced the term of chemoprevention in the early 1970s and defined it as the process of inhibition, suppression or reversion of cancer development or progression by using any natural or synthetic agents[30]. Carcinogenesis is a complex event, and its development is a multistage process that consists of several phases: initiation, promotion, progression and lastly metastasis[31]. For example, lung carcinogenesis derives from multiple steps involving a series of genetic and epigenetic alterations in pulmonary epithelial cells that lead to the changes in cell proliferation, differentiation, invasion, and metastasis[32].

Moreover, lung carcinogenesis also showed the characteristic of "field of cancerization" that refers to the adjacent tissue to neoplastic lesions that appear histologically normal, but this

tissue consists of molecular abnormalities as in the tumours[33]. Example of the "field of cancerization" during lung carcinogenesis is the formation of premalignant or injury in all airway of epithelial cells after exposure to the cigarette smoke[34, 35]. This concept is further supported because of a single mutant epithelial cell in respiratory lining potentially to expand and extend into surrounding tissues in lung and lastly turns into malignancy. Even after smoking cessation, the expansion of premalignant field can still occur, and it is the vital step in lung carcinogenesis[36]. Therefore, effective chemoprevention strategies are urgently needed as lung cancer risk is persistently increased even after the smoking cessation (former smokers)[37].

Natural agents such as phytochemicals are selectively targeting cancer cells, and even in small doses amount, the chemopreventive phytochemicals regularly act on cancer cells without affecting healthy cells[38]. The natural agents from fruits and vegetables have shown several chemopreventive potentials in lung cancer. For example, one study revealed that the consumption of fruits and vegetables in current smokers could reduce the risk of getting lung cancer[39]. Moreover, another study also showed the same finding where the higher consumption of cruciferous vegetables together with cigarette smoking control could lower the risk of lung cancer[40]. Accordingly, there must be many potential candidates from natural sources that can be developed as cancer therapeutic and preventive agents to reduce the adverse effects of conventional cancer treatment.

Non-small cell lung cancer (NSCLC)
Lung Adenocarcinoma (AD)

Lung AD is a type of cancer that develops or arises from epithelial that line the small peripheral airways and categorised under the subtype of non-small cell lung cancer (NSLC). It is the most common type of all lung cancers[41, 42]; hence lung adenocarcinoma is the most studied among other lung cancers. Amid all types of lung cancer, the incidence rate of lung adenocarcinoma in women is rapidly increased, but it is stabilised in men[43]. Lung adenocarcinoma has a poor prognosis due to late diagnosis usually at the metastatic stage leading to failure in treatment[44]. Although lung AD is the most frequent type of lung cancer seen in non-smokers, it also occurs in smoker patients[45]. Other than that, in a multiracial Asian country,

lung adenocarcinoma is more common in younger patients below the age of 40 years old compared to the older patients that have never smoked[46].

The precursor of lung AD involves various genetic changes with the most common are p53 mutation (50% to 70%), epidermal growth factor receptor (EGFR) kinase domain mutation (10% to 40%), Kristen Rat Sarcoma viral oncogene (KRAS) mutation (10% to 30%), and LBK1 mutation (34%)[47, 48]. Both KRAS and EGFR mutations are rarely found in one tumour[49]. KRAS mutation is more common in smokers with an early stage of lung adenocarcinoma or atypical adenomatous hyperplasia (AAH), and EGFR kinase domain mutation is much more common in nonsmokers with lung adenocarcinoma[47, 50]. Moreover, 78% of lung AD patients had EGFR kinase domain mutation, but only 1.9% of patients had KRAS mutations among East Asian nonsmokers. EGFR plays various roles in a tumourigenic process including proliferation, apoptosis, angiogenesis and invasion[47].

Molecular targeted therapy such as targeted kinase inhibitors by gefitinib (Iressa) was performed in lung adenocarcinoma treatment, and this drug has been approved in Japan and the United States[51]. In contrast, there is still no treatment targeting the KRAS mutations. As one of the most common genetic defects in lung AD, KRAS mutations can be the potential genetic abnormality to be targeted in the treatment of lung AD[52]. There have been are many studies reported on the potential of natural agents to give effects as anti-cancer and chemoprevention on lung adenocarcinoma with various underlying mechanisms. Table 2 shows the summary of chemoprevention and chemotherapeutic recent researches on natural products against lung AD including the mechanisms of action.

Squamous cell carcinoma (SCC)

Lung squamous cell carcinoma is an abnormal growth of cells from the bronchial epithelial cells through hyperplasia/metaplasia with the most common features are keratin pearls and/or intercellular bridges[65]. The development of lung SCC is due to inhaled carcinogens that directly expose to the respiratory epithelium, and it is a sequential process that starts with squamous metaplasia, dysplasia, and carcinoma in situ[66]. Historically, lung SCC is the most common type of

lung cancer, but recently the lung adenocarcinoma has replaced the lung SCC as the most common type of lung cancer [12]. Even though the reasons behind this trend is unknown, yet there are several possible factors such as diagnostic advances, changing from high-tar to low-tar filtered cigarettes and variations in smoking patterns[67]. Cigarette smoking has a connection with all histologic types of lung cancer. However, a study done in 2001 revealed that smoking associated strongly with the lung SCC cases as compared to the carcinogenesis of lung adenocarcinoma[68]. This strong association

of smoking and lung SCC also has been proven by another study, where 96% of lung SCC patients in North America were ex-smokers or smokers[69].

None of the targeted therapies for lung SCC has been approved due to the lack of genomic understanding. Moreover, genomic studies on lung SCC is only now emerging, unlike multiple targeted therapies that have been identified in lung adenocarcinoma[70]. For example, the therapeutic approaches that targeted on EGFR and echinoderm microtubule-associated protein like 4 – anaplastic lymphoma kinase (EML4-ALK) are

Table 1. 8th edition of the American Joint Commission on Cancer TNM staging system for non–small cell lung cancer(NSCLC) and the explanation for abbreviation of each stage (19).

Staging	TNM system (T: Primary tumor; N: Lymph node; M: Distant metastasis).
IA1	-N0 with any T1a
IA2	-N0 with T1b
IA3	-N0 with T1c
IB	-N0 with T2a
11A	-N0 with T2b
11B	-N0 with T3-N1 with any T1a, T1b, T1c, T2a, or T2b.
IIIA	-N0 with T4. -N1 with any T3 or T4-N2 with any T1a, T1b, T1c, T2a, or T2b.
IIIB	-N2 with any T3 or T4.-N3 with any T1a, T1b, T1c, T2a, or T2b.
IIIC	-N3 with any T3 or T4
IVA	-Any N0 to N3 with any M1a or M1b
IVB	-Any N0 to N3 with M1c

Explanation

1) Primary tumour(T)

T1a	Tumour in central airway but only superficial spreading (tumor equal or less than 1cm).
T1b	Tumour more than 1 cm but less than or equal to 2cm.
T1c	Tumour more than 2 cm but less than or equal to 3cm.
T2a	Involvement of visceral pleura, main bronchus, atelectasis to hilum, and no carina involvement. Tumour more than 3cm but less than or equal to 4cm.
T2b	Involvement of visceral pleura, main bronchus, atelectasis to hilum, and no carina involvement. Tumour more than 4 cm but less than or equal to 5cm.
T3	Invasion of tumour to the chest wall, pericardium, phrenic nerve; or one or more tumor nodules in same lobe with primary tumour; or tumour more than 5 cm but less than or equal to 7cm.
T4	Invasion of tumour to the mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, esophagus, and spine; or tumor nodules in different ipsilateral lobe from where the primary tumor located; or tumour more than 7cm.

2)Lymph node(N)

N0	No of regional lymph node metastasis.
N1	Involvement of primary tumour that metastasis to the ipsilateral pulmonary or hilar lymph nodes.
N2	Involvement of primary tumour that metastasis to the mediastinal or subcarinal lymph nodes.
N3	Involvement of primary tumour that metastasis to the contralateral mediastinal, hilar or supraclavicular lymph nodes.

3) Distant metastasis(M)

M1a	Metastasis of primary tumour to the pleural or pericardial; or metastasis of tumor to the contralateral lobe from primary tumor.
M1b	Metastasis of tumour to any single extrathoraric.
M1c	Metastasis of tumour to multiple extrathoraric (1 or more organs).

Table 2. The summary of chemoprevention and chemotherapeutic effects of natural products against lung AD (53-64).

Natural product	Sources	Mechanisms of action	Reference
Chemoprevention			
Pterostilbene	*Vitis vinifera* leaves and fruits; blueberries and cranberries	*In vivo:* • Pterostilbene decreased the epidermal growth factor receptor EGFR expression and also decreased the expression of other EGFR mediators such as Akt/mTOR, ERK1/2, Stat-3, and NFκB.· • Pterostilbene also induced apoptosis by decreasing the level of caspase-3 and LC3-II.	Chen et al. 2012 (53).
β-escin	Horse chestnut seeds (*Aesculus hippocastanum*).	*In vivo:* • Reduced the number of tumours in a mouse model. â-escin induced cell cycles arrest by reducing the expression of ALDH1A1, p-Akt, RhoA, and ROCK proteins. However, it increased the expression of p21 protein and reduced the level of PCNA.	Patlolla et al. 2013(54).
Cucurbitacin B (CuB)	Cucurbitaceae plants such as fruit of L.graveolense Roxb.	*In vitro:* • CuB induced cell cycle arrest at G2/M phase and apoptosis. *In vivo:* • Reduced the tumour multiplicity and PCNA-positive cells. *In vitro and in vivo:* • Upregulation of p16 and p21 proteins expression and downregulation of tumour suppressor proteins such as c-myc, KRAS, and hTERT. • CuB inhibited the expression of DNMTs and histone deacetylase (HDACs).	Shukla et al. 2015 (55).
Chemotherapeutic			
Curcumin	Rhizome of *Curcumalonga.*	*In vitro:* • Curcumin treatment inhibited human lung adenocarcinoma derived cells (H441 cells) by suppressing the activation of the Stat-3 pathway.	Alexandrow et al. 2012 (56).
Honokiol	*Magnolia grandiflora.*	*In vitro:* • Inhibited cell growth and induced cell cycle arrest at the G1 phase of A549 and H1299 cell lines. • Repressed histone deacetylase (HDAC) activity and class 1 HDACs protein expression, but increased the activity of HATs. *In vivo:* • Inhibited tumor growth of A549 and H1299 cell lines in a xenograft model. Induced apoptosis by downregulating Bcl-2 and Bcl-xl proteins expression. • The levels of class I HDACs and HDACs activity also decreased in this in vivo study.	Singh et al. 2012 (57).
Emodin	Roots and rhizomes *Rheum palmatum* L.	*In vitro:* • Emodin suppressed the proliferation of A549 cell lines by activation of ERK1/2 and downregulation of ERCC1 and Rad51 proteins expression that led to cytotoxicity.	He et al. 2012 (58).
Diosein	The roots of *Polygonatum zanlanscianense* Pamp.	*In vitro:* • Inhibited proliferation of A549 cell lines by causing DNA damage and cell cycle arrest at S phase. • Diosein induced apoptosis by upregulating the pro-apoptotic proteins expression (Bax, Bak, and Bid). Downregulated the anti-apoptotic proteins expression (Bcl-2 and Bcl-xl). Increased the caspase-3 and caspase-6 activities.	Wei et al. 2013 (59).
Piperine	Black pepper (*Piper nigrum*) and long pepper (*Piper longum*).	*In vitro:* • Piperine inhibited the proliferation of A549 cell lines by causing cell cycle arrest at the G2/M phase via p53 dependent mitochondrial signalling pathway. • Induced apoptosis by activating caspase-3	Lin et al. 2014 (60).

		and caspase-9, increased BAX and p53 proteins expression and downregulated Bcl-2 protein expression.	
Plumbagin (PL)	*Plumbago zeylanica* L.	*In vitro:* • Inhibited proliferation of A549 and H23 cell lines by causing apoptosis and cell cycle arrest at G2/M phase.· • Cell cycle arrest by downregulating the expression of cyclin B1 and Cdc2, but upregulated the expression of p53 and p21.· • Induction of apoptosis by downregulating the expression of Bcl-2 and upregulating the expression of Bax and cytochrome c. Increased the levels of cleaved caspase-3 and 9.· • PL also increased the reactive oxygen species (ROS) production.· • Inhibited PI3K/Akt/mTOR pathway to induce autophagy as well.	Li et al. 2014 (61).
TXA9	The roots of *Streptocaulon juventas*.	*In vitro:* • Induced apoptosis by increasing the level of Fas protein, Fas-associated death domain (FADD) and enzymes caspase-3 and caspase-8. *In vivo*: • Inhibited tumour growth in a xenograft model.	Xue et al. 2015 (62).
Epigallocatechin -3-gallate (EGCG)	Green tea	*In vitro:* • EGCG inhibited nicotine-induced migration and invasion of A549 cells by suppressing the level of HIF-1á, vascular endothelial growth factor (VEGF), COX-2, p-Akt, p-ERK and vimentin proteins expression. • EGCG also downregulated expression of p53 and β-catenin proteins.	Shi et al. 2015 (63).
Picropodophyllin.	Mayapple plant family (*Podophyllum peltatum*).	*In vitro:* • Inhibited proliferation of A549 and H1299 cell lines by repressing the expression of insulin-like growth factor 1 (IGF-1R) and decreasing the p-Akt and MAPK. Induced apoptosis by increasing the level of caspase 3, 7 and PARP proteins.	Zhang et al. 2015 (64).

*EGFR= epidermal growth factor receptor; Akt/mTOR = protein kinase B/mammalian target of rapamycin (mTOR); ERK 1/2= extracellular-signal-regulated kinase; ALDH1A1= aldehyde dehydrogenase 1A1= ALDH1A1; Stat-3= signal transducer and activator of transcription3; LC3-II= microtubule-associated proteins 1A/1B light chain 3B-11; p-AKT= phospho-Akt; RhoA= Ras homolog gene family member A; ROCK= Rho-associated protein kinase; p21= cyclin-dependent kinase inhibitor 1; hTERT= human telomerase reverse transcriptase; KRAS= Kristen rat sarcoma 2 viral oncogene homolog; DNMTs= DNA methyltransferase; HDACs= histone deactylases; Stat-3= signal transducer and activator of -3; HDAC= histone deacetylase; HATs= histone acetyltransferases;Bcl-2= B-cell lymphoma 2; Bcl-xl= B-cell lymphoma-extra large; ERK 1/2= extracellular-signal-regulated kinase; ERCC1=excision repair cross-complementation group 1; RAD51= RAD51 recombinase; BAX=bcl-2-like protein 4; Cdc2=cyclin-dependent kinase 1; PI3K=phosphoionositide 3-kinase;FADD=Fas-associated protein with death domain; HIF-1á=hypoxia inducible factor 1 alpha; VEGF=vascular endothelial growth factor ; COX-2= cyclooxygenase-2; MAPK=mitogen activated protein kinases; PARP= poly(ADP-ribose) polymerase

only applicable in lung adenocarcinoma treatment, but not in lung SCC[71]. For example, a clinical study in 2006 reported that bevacizumab that acts as endothelial growth factor (VEGF) inhibitors together with standard treatment (platin-based chemotherapy) increased the survival of NSCLC patients. The Eastern Cooperative Oncology Group (ECOG) performance status of patients with advanced NSCLCs also better compared to the standard treatment alone without bevacizumab. Unfortunately, the results are different for lung SCC patients, where there is no improvement in survival and ECOG performance status in lung SCC patients[72]. Thus, the treatment that effective for lung adenocarcinoma does not promise the same results in lung SCC. More studies on lung SCC is needed due to the limited progression in lung SCC treatment as compared to lung adenocarcinoma, even though both are the most common types of lung cancer.

The most common genetic changes in lung SCC is the mutation of TP53 gene with 60 to 70% of lung SCC cases had this mutation, followed by phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) amplification that accounted for 33% of lung SCC cases[47]. Apart from the alterations of TP53 and PIK3CA, Cancer Genome Atlas Research Networks 2012 revealed the list of most common genetic alterations in lung SCC including the mutations of histone-lysine N-methyltransferase 2D(MLL-2), cyclin-dependent kinase 2A (CDKN2A), nuclear factor

erythroid 2 like2 (NFE2L2), Kelch-like ECH-associated protein 1(KEAP1), and others. Others common genetic changes or alterations in lung AD such as mutations of EGFR and KRAS and fusion of anaplastic lymphoma kinase(ALK), but all these types of genetic changes are infrequent in lung SCC cases[71]. Table 3 shows the the summary

of chemoprevention and chemotherapeutic recent researches on natural products against lung SCC including the mechanisms of action.

Large cell carcinoma (LCC)

Large cell lung carcinoma (LCC) is a poorly differentiated subtype that often arises in peripheral lung tissue[77]. In 2013, a team of

Table 3. The summary of chemoprevention and chemotherapeutic effects
of natural products against lung SCC (58, 64, 73-76).

Natural products	Sources	Mechanisms of action	Reference
Chemoprevention			
Honokiol	Bark of Magnolia tree.	*In vivo:* • Honokiol interrupted the mitochondrial function by causing inhibition of mitochondrial respiration leading to a decreased in ATP levels. The decreased in ATP levels activated the AMPK and lastly inhibited the tumour growth. • Honokiol enhances the ROS production in mitochondria.• Induction of apoptosis by increasing the level of cleaved caspase-3.	Pan et al. 2014(73).
Picropodophyllin.	Mayapple plant family (*Podophyllum peltatum*).	*In vivo:* • Reduced the tumour load and multiplicity in benzo(a)pyrene-induced lung tumours in a mouse model by increasing the levels of cleaved caspase-3 apoptotic protein.	Zhang et al. 2015 (64).
Vitamin D3	.	*In vivo:* Vitamin D3 inhibited the proliferation rate by reducing the levels of Ki67 protein.• Vitamin D3 induced the anti-inflammatory activity by reducing the expression of IL-6, and the white blood cell (WBC) counts.	Mazzilli et al. 2015 (74).
Chemotherapeutic			
Emodin	Roots and rhizomes *Rheum palmatum* L.	In vitro: • Emodin inhibited the proliferation of SK-MES-1 cell lines (human lung squamous carcinoma cells). Emodin activated the ERK1/2 and downregulated the expression of ERCC1 and Rad51 proteins that caused cytotoxicity.	He et al. 2012 (58).
Alantolactone	*Inula helenium* L. roots.	*In vitro:* • Inhibited SK-MES-1 cells growth by causing apoptosis and cell cycle arrest at the G0/G1 phase.• Apoptosis- Downregulated the expression of Bcl-2, procaspase 9 and 3. Upregulated the expression of Bax, cleaved caspase-3, and PARP. • Cell cycle arrest- Downregulated the expression of CDK4, CDK6, cyclin D3, and cyclin D1. Upregulated the expression of p21 and p27.	Zhao et al. 2015 (75).
Custonolide	*Compositae* and *Magnoliaceae* plant families.	*In vitro:* • Induced cell cycle arrest at the G1/S phase and apoptosis in SK-MES-1 cell lines.• Apoptosis by downregulating Bcl-2 and procaspase-3 expression and upregulating Bax and cleaved PARP proteins expression.• Caused cell cycle arrest by upregulating p53, p21, and p27 expression, and downregulating pRB proteins.	Hua et al. 2016 (76).

*ATP= adenosine triphosphate; AMPK= 5' AMP-activated protein kinase; Ki-67cell proliferation antigen Ki-67; IL-6 = interleukin-6; ROS=reactive oxygen species. ERK1/2=extracellular signal-regulated kinases 1/2; Rad51=DNA repair protein Rad51;ERCC1=DNA excision repair protein ERCC-1; CDK4=cyclin dependent kinase -4; CDK6; cyclin dependent kinase-6;pRB=phosho-retinoblastoma protein; p21=cyclin dependent kinase inhibitor 1; p53=tumor suppressor p53; p27cyclin dependent kinase inhibitor 1B.

Table 4. The summary of chemotherapeutic researches of natural products
against lung LCC and SCLC (59, 82, 83, 94-97)

Natural products	Sources	Mechanisms of action	Reference
Lung large cell carcinoma (LCC)			
Didymin	Flavonoid glycoside from citrus fruits.	*In vitro:* • Inhibited H460 cells growth by inducing apoptosis. · • Induced apoptosis by down regulating the expression of Fas/Apo-1 receptor and Fas-ligand. Didymin also activated the caspase-8.	Hung et al. 2010 (82).
α-tomatine	*Lycopersicones culentum* Linn.	*In vitro:* · • α-tomatine showed anti-metastatic effects on NCI-H460 cell lines. · Reduced the mRNA level and MMP-7 protein expression. · • α-tomatine suppressed degradation and phosphorylation of DNA-binding activity of NF-êB and FAK/PI3K/ Akt signalling pathway.	Shieh et al. 2011 (83).
Diosein	*Polygonatum zanlanscianense* Pamp	*In vitro:* • Inhibited proliferation and induced apoptosis in NCI-H460 cells. • Inhibited cell growth by causing DNA damage and cell cycle arrest at the S phase. · • Apoptosis: upregulated the expression of pro-apoptotic proteins (Bax, Bak, and Bid). • Downregulated the expression of anti-apoptotic proteins (Bcl-2 and Bcl-xl). • Increased the caspase-3 and caspase-6 activities.	Wei et al. 2013 (59).
Small cell lung cancer(SCLC)			
Curcumin	Rhizome of *Curcumalonga*	*In vitro:* · • Inhibited cell proliferation, migration and invasion of human small cell lung carcinoma (NCI-H446 cell line). · • Curcumin downregulated the expression of STAT3 by inhibiting the IL-6 leading to the inhibition of STAT3 phosphorylation. · • Induced cell cycle arrest at the G2/M phase by downregulating the expression of Survivin, Bcl-xL and Cyclin B1. · • Depressed the migration and invasion of cells by suppressing the levels of VEGF, MMP-2 and MMP-7 and ICAM-1.	Yang et al. 2012 (94)·
Catechins	Green tea	*In vitro*: • Caused cell cycle arrest at the G2/M phase of NCI-H466 cell lines. · • Upregulated the expression of let-7a-1 and let-7g protein leading to the downregulation of C-MYC and LIN-28 expression and induced cell cycle arrest.	Zhong et al. 2012 (95).
Diosein	The root of *Polygonatum zanlanscianense* Pamp	*In vitro:* • Inhibited cell proliferation and induced apoptosis in NCI-H446 cells. · • Anti-proliferation: induced DNA damage and cell cycle arrest at the S phase. · • Apoptosis: upregulated the expression of pro-apoptotic proteins (Bax, Bak, and Bid). Downregulated the expression of anti-apoptotic proteins (Bcl-2 and Bcl-xl).	Wei et al. 2013 (59).

		Diosein also increased the caspase-3 and caspase-6 activities.	
Wentilactone A (WA)	Marine-derived endophytic fungi *Aspergillus wentii.*	*In vitro*: • Induced apoptosis and cell cycle arrest at the G2/M phase in NC1-H446 cell lines. • Induced cell cycle arrest by downregulating the expression of cyclin B1, cdc2, p-cdc2, cdc25C and p-cdc25C. WA increased the phosphorylation of ERK, JNK, p38 to activate the mitogen-activated protein(MAP) kinase signalling cascades. WA also induced the expression of p53, p21 and ROS production to induce cell cycle arrest.· • Apoptosis- Downregulated the expression of Bcl-2 and Mcl-1, but upregulated the expression of Bax, Bad, cleaved caspase-2 and caspase 7.	Lv et al. 2013 (96).
Evodiamine	*Evodia rutaecarpa*	*In vitro:* • Induced cell cycle arrest at the G2/M phase and apoptosis in H446 and H1688 cell lines.· • Increased the ROS production and activities of caspase 8, 9 and 3.· • Increased the expression of cytochrome C, caspase 3, 8, 9, and Bax proteins, and decreased the expression of Bcl-2 protein.	Fang et al. 2014 (97).

*Apo-1=Apoliprotein A1; MMP-7= matrix metalloproteinase-7; NF-kâ= nuclear factor kappa-light-chain enhancer of activated B cells; FAK= focal adhension kinase; PI3K= phosphatidylinositol 3-kinase; Akt= protein kinase B; Bax= Bcl-2 associated X protein; Bak= Bcl-2 homolog antagonist/killer; Bid=BH3 interacting-domain death agonist; Bcl-2= B cell lymphoma 2; Bcl-xL= B cell lymphoma extra large. STAT3= Signal transducer and activator of transcription 3; IL6+interleukin 6; VEGF=vascular endothelial growth factor; MMP=matrix metalloproteinase; ICAM-1= intracellular adhension molecule 1; LIN28= Lin-28 homolog A protein; cdc25c= M-phase inducer phosphatase 3; ERK= extracellular-signal regulated kinase; JNK= c-Jun N-terminal kinases; ROS= reactive oxygen species; Bax= Bcl-2 associated X protein; Bak= Bcl-2 homolog antagonist/killer; Bid=BH3 interacting-domain death agonist; Bcl-2= B cell lymphoma 2; Bcl-xL= B cell lymphoma extra large; Bad; Bcl-2 –associated death promoter

researchers revealed that the tumour site of lung LCC is mostly located at the lung periphery with 81 cases (67%) out of 121 casesalso showed that LCC cases have a strong relation with smoking habit in which about 92% of patients are smokers or former smokers[78].

Large cell neuroendocrine carcinoma (LCNC) is one example of lung large cell carcinoma (LCC), that shares the same characteristics with small cell lung cancer (SCLC) such as massive proliferation rates and neuroendocrine phenotype but lacks cytomorphology of SCLC[79, 80]. LCNC is a rare type of lung cancer with estimated to account only for 3% of all lung cancers[81]. The most common genetic changes in LCNC is the alteration of TP53 that accounts for 78% of LCNC cases. Others common genetic changes in LCNC including the alteration of few genes that encoded for retinoblastoma (RB1) protein,

serine/threonine kinase II (STK II), Kelch-like ECH-associated protein 1 (KEAP1), and KRAS[80]. Chemoprevention of lung LCC was excluded from the discussion due to limited chemopreventive study on lung LCC. Besides being a less common type of lung cancer, the limited chemopreventive study of lung LCC might be due to difficulties of developing the animal model that relevance to the human lung LCC. Table 4 shows the summary of chemotherapeutic researches of natural products against lung LCC and SCLC.

Small cell lung cancer (SCLC)

Small cell lung cancer (SCLC) is a type of lung cancer that develops from neuroendocrine-cell precursors. Some essential characteristics of SCLC are rapid cell growth, highly vascularized, genomic instability and early onset of metastatic. SCLC has the lowest survival rate among all type of lung cancers with only 3% to 8% of five-year survival

rate, but the five-year survival rate of other types of lung cancers are more than 15% (84). Most of the SCLC patients are diagnosed with metastatic as the cancerous cells disseminated rapidly through the blood and lymphatic system[85]. The response rates of SCLC towards both chemotherapy and radiotherapy is high, yet, patients with metastatic SCLC tends to develop treatment resistance[86, 87]. The high risk of SCLC has a strong connection with cigarette smoking. Smoking cessation has an enormous impact on SCLC by reducing the risk of getting SCLC and increasing the survival rate among the patients with localised SCLC by almost 50%[88]. Moreover, a study found that among 148 SCLC patients only 3 patients were non-smokers and another 145 patients were smokers[89].

The most common molecular genetic changes in SCLC are overexpression of BCL-2 and inactivation of p53 and retinoblastoma (Rb) protein. Inactivation of p53 and Rb protein has been found to account up to 90% of SCLC cases, and the inactivation of the two proteins can be caused by a mutation (gene deletion)[90]. Other than differences in histological features between SCLC and NSCLC, these two subtypes of lung cancer also different in term of expression of neuroendocrine (NE) differentiation markers. NE differentiation markers only express in SCLC but not in NSCLC such as chromogranin A, neuron-specific enolase, synaptophysin, or neural cell adhesion molecule[91, 92]. Study on chemoprevention of SCLC is minimal, especially on chemopreventive effects of natural compounds toward SCLC. Difficulties in developing a mouse model that relevance to human SCLC can be due to the complexity of this type of lung cancers such as SCLC tumors contain different tumor type of cell populations (cells heterogeneity)[93]. Thus, the chemoprevention against SCLC was excluded from this review. Table 4 shows the summary chemotherapeutic researches of natural products against SCLC together with LCC.

CONCLUSION

Since numerous interventions have been done to improve the survival rate and burden of lung cancer patients, but lung cancer is still one of the major causes of cancer-related death. Other than focusing on improvement of

treatment, chemoprevention of lung cancer has gained increasing popularity lately. Molecular targeted therapy specifically on each subtype of lung cancers might be one of the alternatives to improve the current treatments as it provides high efficacy approaches. Natural products are a great potential candidate for chemotherapeutic and chemoprevention as they have less harmful side effects and adverse reactions. Therefore, the discovery of new chemotherapeutic and chemopreventive agents are urgently needed to control lung cancer cases. This review of findings from in vitro and in vivo studies using human cell lines and experimental animal model specifically on each subtype of lung cancers may be a good source for further investigation, including the clinical study to discover the effective chemotherapeutic and chemoprevention agents for lung cancer.

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