Proanthocyanidins and Anthocyanins in Nicotine-Induced NSCLC Treatment

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In traditional medicine, different parts of plants, including fruits, have been used for their anti-inflammatory and anti-oxidative properties. Plant-based foods, such as fruits, seeds and vegetables, are used for therapeutic purposes due to the presence of flavonoid compounds. Proanthocyanidins (PCs) and anthocyanins (ACNs) are the major distributed flavonoid pigments in plants, which have therapeutic potential against certain chronic diseases. PCs and ACNs derived from plant-based foods and/or medicinal plants at different nontoxic concentrations have shown anti-non-small cell lung cancer (NSCLC) activity in vitro/in vivo models through inhibiting proliferation, invasion/migration, metastasis and angiogenesis and by activating apoptosis/autophagy-related mechanisms.

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1. Introduction

Lung cancer (LC) is considered the main diagnosed cancer causing death worldwide [1]. LC is broadly categorized into two major histologic classes: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is further subclassified into squamous, adenocarcinoma and large cell carcinoma, which constitute 85% of smoking-attributable LC cases [2][3]. Tobacco products contain several carcinogenic compounds, such as tobacco-specific nitrosamines (i.e., NNN and NNK), which enhance production of DNA adducts in the lungs of smokers, thereby causing mutations of several NSCLC suppressor genes including protein p53 [4][5][6]. Although nicotine is considered non-carcinogenic, it may contribute to NSCLC development [7][8][9][10][11]. Nicotine enhances proliferation, angiogenesis and metastasis and inhibits apoptosis/autophagy in NSCLC cells by activating nicotinic acetylcholine receptors (nAChRs), especially the α7 subunit, and its downstream signaling pathways including the proto-oncogene serine/threonine kinase (Rb-RAF1), the phosphatidylinositol-3 kinase/serine/threonine kinase (PI3K/Akt), the mammalian target of rapamycin (mTOR), the nonreceptor tyrosine/kinase focal adhesion/protein kinase (Src/FAK/PKC) and the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) [12]. Nicotine also induces epithelial-to-mesenchymal transition (EMT), which is the key step in enhancing tumor progression in NSCLC cells, resulting in upregulation of several transcription and growth factors via activation of α7nAChR-mediated signaling pathways [12]. The mechanisms by which nicotine enhances tumor progression in NSCLC cells have been previously described in greater details [13]. In brief, nicotine binds to α7nAChR, activating the cellular signaling pathways involved in proliferation, metastasis, angiogenesis and anti-apoptosis/autophagy, which increases expression of EMT-associated molecules in NSCLC cells such as hypoxia inducible factor-1 (HIF-1α), vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), deca-pentaplegic homolog

(Smad), B-cell lymphoma-2 (Bcl-2), metalloproteinases (MMPs), cyclinD1, Snail, twist, vimentin, fibronectin and N-cadherin [13]. **Figure 1** summarizes the mechanisms for nicotine in the development of NSCLC.

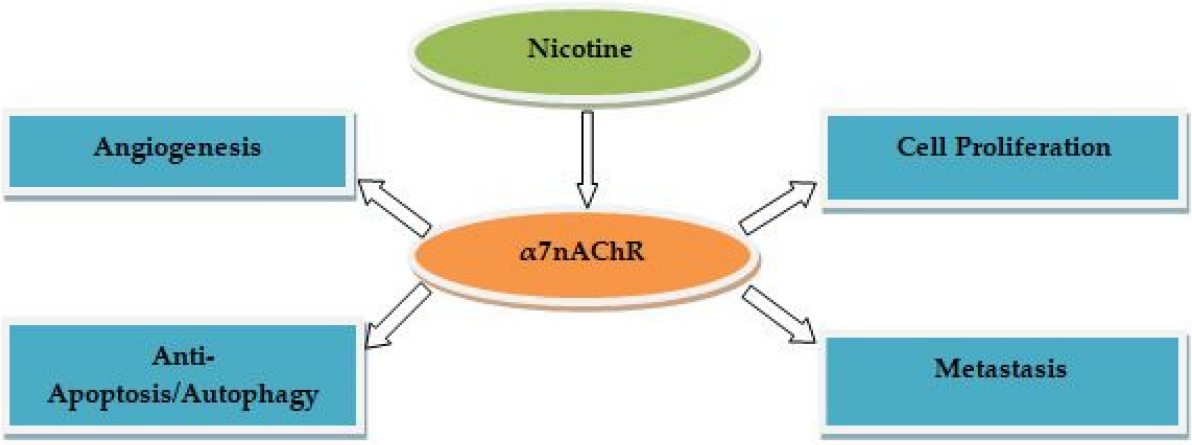

Figure 1. Mechanisms for nicotine in the development of NSCLC.

There is no clear recommendations on safe dietary supplements for use in treating NSCLC, particularly in smokers [14]. However, combination therapy of monoclonal antibody-based immunotherapy and chemotherapeutic agents has been shown to be a promising treatment strategy for NSCLC [15]. In addition, natural flavonoids present in fruits (e.g., citrus) in combination with chemotherapeutic agents, such as cisplatin, have shown anti-NSCLC effects, demonstrated by inhibition of the α7nAChR-mediated signaling pathways involved in cellular processes including proliferation, inflammation and anti-apoptosis [13][16].The health benefits of fruits are due to the high levels of bioactive flavonoids they contain, such as proanthocyanidins (PCs) and anthocyanins (ACNs) [17][18]. A large number of studies have documented the health benefits of natural flavonoids derived from fruits and plant foods, in relation to their biological attributes such as anti-diabetes/anti-cancer activity, reducing cardiovascular diseases and improving the blood lipid profile [17][18][19]. Thus, there is a real need to investigate natural flavonoid compounds, such as PCs and ACNs, as therapeutic agents for nicotine-induced NSCLC.

Flavanols include a group of natural compounds categorized according to their chemical structures into catechins and PCs, which are found in various plant-based foods. PCs, also known as condensed tannins, are polymeric and/or oligomeric pigments found in common plant-based foods (e.g., fruits, vegetables, cereal grains, and legumes), Cinnamomi Cortex (barks of *Cinnamomum cassia* used as a traditional Chinese medicine) and *Vaccinium* berries, for which a range of therapeutic effects have been reported including anti-cancer, antimicrobial, anti-diabetic, anti-obesity, cardioprotective and antioxidant properties [20]. Procyanidins are the most homo-oligomeric PC derivatives comprised of epicatechin/catechin monomeric, connected via the C4→C6/C4→C8 bond (B-type linkage) and the C2→O7 bond (A-type linkage), and dominated by dimers (e.g., procyanidin A1-A2/B1-B8), trimers (e.g., selligueain A/B and procyanidin C1/C2), and tetramers (degree of polymerization ranged from 5 to 11) [20][21][22].

ACNs, featuring six common glycosylated forms of anthocyanidins (i.e., malvidin, pelargonidin, cyanidin, petunidin, delphinidin and peonidin) with hydroxyl (OH) moieties in their structure at the 3 position on the C-ring are water-

soluble pigments belonging to flavonoids responsible for producing various colors in fruits, berries and vegetables that exert a protective effect against diabetes, cardiovascular and neurodegenerative diseases and cancers (including LC) [23][24][25].

2. Proanthocyanidins in Nicotine-Induced NSCLC Treatment

Several studies demonstrated the therapeutic effects of PC-rich extracts from plant-based foods and/or medicinal plants at different nontoxic concentration against nicotine-induced NSCLC. Cranberry-derived PCs suppress tumor cell growth in NSCLC cells [26][27], but the mechanisms for this action have not been well investigated [26]. Treatment with cranberry PCs resulted in a significant induction of apoptosis and cell cycle arrest in NSCLC cells via upregulating the expression of pro-apoptotic-related markers (e.g., cytochrome *c* and caspase 3) [28].

PCs from grape seed extract have shown promising results in nicotine-induced NSCLC treatment. For example, using the in vivo proteolysis/antitumor assay and the in vitro proteolysis/angiogenesis assay, PCs inhibit angiogenesis-mediated tumor growth in NSCLC cells, in part by suppressing vascular extracellular matrix (ECM) proteolysis byMMP-2 [29]. Treatment of NSCLC cells with PCs using the in vivo tumor xenograft assay and the in vitro 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for cell proliferation/survival resulted in suppression of cell proliferation in vitro/vivo, and inhibition of angiogenesis and induction the apoptotic cell death of tumor cells in vivo. Such effects are mediated by upregulation of insulin-like growth factor binding protein-3 (IGFBP-3) levels and inhibition of proliferating cell nuclear antigen (PCNA) in the tumor microenvironment [30]. A study used the in vivo tumor xenograft assay and the in vitro cell deathenzyme-linked immunosorbent assay (ELISA) and MTT assay for assessing proliferation of NSCLC cells showed that PCs cause proliferation inhibition and apoptosis induction via the inhibition of cyclooxygenase-2 (COX-2) expression and prostaglandin-2 (PGI-2) receptors in NSCLC cells [31]. The mechanism underlying the anti-migration effect of PCs on NSCLC cells involve inhibiting nitric oxide (NO) synthase, N(G)-nitro-L-arginine methyl ester (L-NAME), and the ERK1/2 and MAPK signaling pathways [32]. The anti-proliferative/apoptotic effects of PCs onNSCLC cells are mediated via the activation of caspase 3 expression, prostacyclin synthase (PTGIS)/PGI2 (as measured by 6-keto PGF1α), and 15-lipoxigenase-2/15(S)-hydroxyeicosatetraenoic acid (15-LOX-2)/15-HETE production [33]. PCs showed the inhibitory effects on the cigarette smoke condensate (CSC)-induced migration of NSCLC cells through inhibition of NADPH oxidase (NOX)-induced oxidative stress and EMT transition [34]. Treatment with PCs using the colorimetric caspase-3 activity assay in vivo and in vitro showed apoptotic effects through increased expression of pro-apoptotic markers (e.g., poly ADP ribose polymerase (PARP); Bcl-2-associated X protein (Bax)), and decreased expression of apoptotic markers (e.g., Bcl-2 and cyclins) [35]. A study used the in vivo tumor xenograft assay and the in vitro MTT and miR-106b ISH assays showed that PCs promote anti-proliferative/invasive effects on NSCLC cells via downregulating miR-106b expression and upregulating cyclin-dependent kinase inhibitor 1A (CDKN1A) mRNA and p21 expression [36].

A few studies on NSCLC cells after treatment with the Cinnamomi Cortex extract PCs showed a significant reduction in nuclear factor-E2-related factor 2(Nrf2) expression, and insulin-like growth factor-1 receptors (IGF-1R)

were responsible for induced proliferation [37][38]. Cinnamomi Cortex extract procyanidin C1 exert anti-metastatic activity by suppressing TGF-β-induced EMT in NSCLC cells [39].

Treatment with PCs inhibits hydrogen peroxide (H_2O_2)-induced NSCLC cell viability, as shown by reduced reactive oxygen species (ROS) and malondialdehyde (MDA) production, hydrogen peroxide(H_2O_2)-induced oxidative stress, and promoted the expression of Nrf2 target genes [40]. PCs inhibit proliferation, viability, along with induction of apoptosis and G2/M cell cycle arrest in NSCLC cells. This is triggered by inhibiting the EMT-related molecules (e.g., N-cadherin and vimentin), expression of apoptotic markers (e.g., Bcl-2), and increasing expression of pro-apoptotic markers (e.g., Bax) via downregulating the Janus kinase/signal transducer and activator of transcription3 (JAK2/STAT3) signaling pathway [41].

Treatment with prodelphinidin B-2 3′-O-gallate, a proanthocyanidin gallate, resulted in the upregulation of key transcription factors such as the soluble Fas ligand (sFasL) and membrane-bound Fas ligand (mFasL), which are responsible for the anti-proliferative and apoptotic activities in NSCLC cells [42][43]. Cinnamtannin D1, an A-type procyanidin trimer, from *Rhododendron formosanum* extracts has been found to exhibit autophagic effects on NSCLC cells via inhibition of cellular signaling pathways (e.g., mTOR) [44].

These results suggest that plant-derived natural PCs may play a significant role as anti-NSCLC agents by suppressing proliferation, migration, invasion, viability, metastasis, angiogenesis, and promoting apoptosis/autophagy via inhibition/activation of transcription factors and/or multiple cellular signaling pathways induced by α7nAChR in NSCLC cells. **Table 1** highlights the molecular mechanisms of PCs in nicotine-induced NSCLC treatment.

Table 1. The molecular mechanisms of PCs in nicotine-induced NSCLC treatment.

Study Type	NSCLC Cell Type	Extract/Compound	Concentrations	Activity	Mechanisms of Action	Reference
In vitro	DMS114	Cranberry presscake	200–300 μmol/L	Anti-proliferative, apoptosis	NA	[26]
In vitro	H460	Cranberry (*Vaccinium macrocarpon*)	20–80 μg/mL	Anti-proliferative	MMP2, MMP9↓	[27]
In vitro	H460	Cranberry (*Vaccinium macrocarpon*)	50 μg/mL	Apoptosis, cell cycle arrest	P21, P73, PARP, cytochrome *c*, caspase3/4/8↑ Bcl-2↓	[28]
In vitro/vivo	A549	Grape seed	100 μg/mL (in vitro) 30 mg PC/kg	Inhibition of tumor angiogenesis	MMP2↓	[29]

Study Type	NSCLC Cell Type	Extract/Compound	Concentrations	Activity	Mechanisms of Action	Reference
			bodyweight (in vivo)			
In vitro/vivo	A549, H1299	Grape seed	60, 80 μg/mL (in vitro) Administration of PCs (0.1%, 0.2%, and 0.5%, bodyweight) (in vivo)	Anti-proliferative, anti-angiogenic, apoptosis	PCNA↓ IGFBP-3↑	[30]
In vitro/vivo	H157, H226, H460, H1299, A549	Grape seed	20, 40, and 60 μg/mL (in vitro) Administration of PCs (0.5%, bodyweight) (in vivo)	Anti-proliferative, apoptosis	COX-2, PGI-2↓	[31]
In vitro	A549, H1299	Grape seed	10, 20, 40, and 60 μg/mL	Anti-migration	NO, L-NAME, MAPK, ERK1/2↓	[32]
In vitro	A549	Grape seed	6 μg/mL	Anti-proliferative, apoptosis	caspase 3, PTGIS/PGI2↑	[33]
In vitro	A549, H1299, H460	Grape seed	20 and 40 μg/mL	Anti-migration	E-cadherin, NOX, p22/p47(phox)↓ N-cadherin, fibronectin, vimentin↑	[34]
In vitro/vivo	A549, H1299	Grape seed	20, 40, and 60 μg/mL (in vitro) 50, 100, and 200 mg PC/kg bodyweight (in vivo)	Apoptosis	G1arrest, Bax, caspases-3/9, Cdki, PARP↑ Bcl-2,Bcl-xl, Cdk2/4/6, cyclins↓	[35]
In vitro/vivo	A549	Grape seed	45 μg/mL (in vitro) 112 mg PC/kg bodyweight (in vivo)	Anti-proliferative, anti-invasive	CDKN1A, p21↑, miR-106b↓	[36]
In vitro	A549	Cinnamomi Cortex	2.5 μg/mL	Inhibition of cell viability	Nrf2↓	[37]

Study Type	NSCLC Cell Type	Extract/Compound	Concentrations	Activity	Mechanisms of Action	Reference
				and proliferation		
In vitro	A549	Cinnamomi Cortex	10 μg/mL	Inhibition of cell proliferation	Nrf2, IGF-1R↓	[38]
In vitro	A549	Cinnamomi Cortex	12.5, 25, 50, and 100 μg/mL	Anti-metastatic	TGF-β, snail, E-cadherin, smad2↓	[39]
In vitro	A549	PCs	≥100 mg/L	Inhibition of cell viability	ROS, MDA, Nrf2↓ HO-1, NQO1, TXNRD1, glutathione, catalase, superoxide dismutase↑	[40]
In vitro	A549	PCs	12.5, 25, 50, 100, and 200 μM	Inhibition of cell viability and proliferation, apoptosis, cell cycle arrest	N-cadherin, vimentin, Bcl-2, MMP2/9, JAK2/STAT3↓, Bax↑	[41]
In vitro	A549	Green tea leaf	1, 5, 10, 20 μM	Anti-proliferative, apoptosis, cell cycle arrest	P21, P53, Fas/sFasL, Fas/APO-1↑	[42]
In vitro	A549	*Myrica rubra*	0.5, 2.5, 5, and 10 μM	Anti-proliferative, apoptosis, cell cycle arrest	Fas/APO-1, P21/WAF1, P53, Fas/sFasL↑	[43]
In vitro	[45] A549, H460	*Rhododendron formosanum*	125, 150, and 175 μM	Autophagy	Akt/mTOR↓	[44]

(↑) increase; (↓) decrease; NA: not mentioned.

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Pulliat inhibit the expression of several transcription and growth factors (e.g., MMP2/9 and VEGF) involved in proliferation, angiogenesis, invasion, and migration of NSCLC cells [46]. The extracts from *Morus alba* L. (cyanidin 3-rutinoside and cyanidin 3-glucoside) inhibit the migratory and invasive activities of NSCLC cells [47]. Cyanidin-3-glucoside not only inhibits the proliferation, invasion, and migration, but also induces apoptosis. The mechanism underlying such an effect is associated with inhibiting p53-induced gene 3 (TP53I3) expression in NSCLC cells and the downregulation of cellular signaling pathways (PI3K/Akt/mTOR) involved in NSCLC progression [48].

Delphinidin inhibits tumor growth, angiogenesis, proliferation, and induces apoptosis in NSCLC cells using the in vivo Matrigel plug assay and the in vitro MTT/ELISA assay by upregulating pro-apoptotic expression (e.g., Bax and

caspase 3/9), along with downregulating epidermal growth factor receptor (EGFR), cobalt chloride (CoCl2)-induced HIF-1α, Bcl-2, PCNA, cyclin D1, and VEGF mRNA expression via inhibiting of several signaling pathways [49][50].

A combination of five ACN extracts (i.e., delphinidin, peonidin, petunidin, cyanidin, and malvidin) from bilberry and blueberry resulted in inhibited growth and metastasis, and induced apoptosis and cell cycle arrest of NSCLC cells in vitro. The mechanism of action of ACNs involves inhibiting activation of multiple signaling pathways, including TNFα-induced NF-$_k$B, Notch, Wnt/β-catenin, and their key transcription factors (i.e., cyclin D1/B1, VEGF, p-ERK, bcl-2, and MMP9). Furthermore, when evaluated with an in vivo model using the in vivo xenograft assay, delphinidin alone, and the ACN mixture, resulted in significantly inhibited growth of H1299 cells [51]. ACNs derived from *Syzygium cumini* L. (known as Indian blackberry) showed significant anti-proliferative effects on NSCLC cells, but the mechanisms for this action have not been observed [52].

These results suggest that ACNs may have a significant role in anti-proliferative, anti-invasive, anti-angiogenic, anti-metastatic, and apoptotic/autophagic effects in NSCLC cells by suppressing the activation of key transcription/growth factors and α7nAChR-mediatedcellular signaling pathways. The molecular mechanisms of ACNs in nicotine-induced NSCLC treatment are summarized in **Table 2**.

Table 2. The molecular mechanisms of ACNs in nicotine-induced NSCLC treatment.

Study Type	NSCLC Cell Type	Extract/Compound	Concentrations	Activity	Mechanisms of Action	Reference
In vitro	A549	*Vitis coignetiae Pulliat*	200 μg/mL	Anti-proliferative, anti-invasive, anti-angiogenic, anti-migration	MMP2/9, cyclin D1, C-myc, COX-2, VEGF↓	[46]
In vitro	A549	*Morus alba* L.	25, 50, and 100 μM	Anti-migration, anti-invasive	MMP2, c-Jun, C-fos, NF-kB↓	[47]
In vitro	A549, H1299	Cyanidin-3-glucoside	5, 10, 20, 40, and 80 μM	Anti-proliferative, anti-migration, anti-invasive, apoptosis	TP53I3 andPI3K/Akt/mTOR↓	[48]
In vitro/vivo	A549, H441, SK-	Delphinidin	5–100 μM (in vitro) 1, 2 mg PC/kg	Inhibition of tumor growth,	EGFR, Bcl-2, PCNA, cyclin D1, VEGFA,	[49]

Study Type	NSCLC Cell Type	Extract/Compound	Concentrations	Activity	Mechanisms of Action	Reference
	MES-1		bodyweight (in vivo)	anti-proliferative, anti-angiogenic, apoptosis	Akt/PI3K/MAPK↓ Bax, caspase-3/9↑	
In vitro/vivo	A549	Delphinidin	10, 20, and 40 μM (in vitro) 80 μM (in vivo)	Anti-angiogenic	EGF, CoCl2, HIF-1α, ERK, VEGF mRNA, Akt/mTOR/PI3K/p70S6K↓	[50]
In vitro/vivo	A549, H1299	Bilberry and blueberry	3.125–12.5 μM (in vitro) 1.5 mg PC/kg bodyweight (in vivo)	Anti-metastatic, anti-invasive, apoptosis, cell cyclearrest	TNFα-induced NF-$_k$B, Notch, Wnt/β-catenin, cyclinD1/B1, VEGF, p-ERK, bcl-2, MMP9 ↓	[51]
In vitro	A549	*Syzygium cumini* L.	2.5, 5, 10, 20, and 25 μM	Anti-proliferative	NA	[52]

(↑) increase, (↓) decrease, NA; not mentioned.

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