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α7 nicotinic acetylcholine receptors in lung cancer (Review)

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Abstract. Lung cancer has one of the highest mortality rates among malignancies globally, and smoking has been documented as the main cause of lung cancer. Nicotinic acetylcholine receptors (nAChRs) were initially identified as notable regulators of the nervous system. In addition to their function in the brain, accumulating evidence indicates that nAChRs perform a host of diverse functions in almost all non-neuronal mammalian cells. The homomeric α7nAChR, a subtype of nAChRs, is responsible for the proliferative, pro-angiogenic and pro-metastatic effects of nicotine in lung cancer. Provided the association of cigarette smoking with several disease types such as cardiovascular disease, the α7nAChR-mediated signaling pathway has been implicated in the pathophysiology of lung cancer. Currently, strategies that target the α7nAChR including α7nAChR antagonists are considered to be potentially useful anticancer drugs for therapeutic purposes. Thus, the present review assesses current understanding of the function and underlying molecular mechanisms of α7nAChR in lung cancer and evaluates how targeting α7nAChR may result in novel therapeutic methods.

Contents

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Abbreviations: nAChRs, nicotinic acetylcholine receptors; NNK, nicotine-derived nitrosamines 4-(methylnitrosamino)-1-(3-pyrydyl)-1-butanone; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; α-CbT, α-cobratoxin

Key words: α7 nicotinic acetylcholine receptor, lung cancer, nicotine, proliferation, angiogenesis, metastasis

1. Introduction

Lung cancer is one of the most commonly occurring carcinoma types globally and has limited treatment options for advanced-stage disease (1). Lung cancer is a heterogeneous disease comprised of two main pathological types: Non-small-cell lung cancer (NSCLC) which accounts for 70-80% of all lung cancer cases and small-cell lung cancer (SCLC) which accounts for ~20% of all lung cancer cases (2). NSCLCs may be divided into three subtypes: Squamous-cell carcinoma (25-30% of all lung cancer cases), adenocarcinoma (~40% of all lung cancer cases) and large-cell carcinoma (10-15% of all lung cancer cases) (3). SCLC is the second most prevalent form of lung cancer, with a 5-year survival rate of <7% (4). Cigarette smoking is considered to be the main risk factor for lung cancer, and ~90% of all cases are associated with exposure to smoking and second-hand smoking (5). Other contributory factors include residential radon, occupational hazards including exposure to asbestos, arsenic and polycyclic aromatic hydrocarbons, radiation, coal smoke, indoor emission of fuel burning, outdoor pollution, previous non-malignant lung diseases in addition to a family history of tumors (6,7). Squamous-cell, large-cell and SCLC are the most commonly identified types of lung cancer present in smokers (8,9). In contrast, adenocarcinoma is the lung cancer type most commonly identified in non-smokers (10).

Cigarette smoke is a mixture of thousands of chemical compounds, a number of which have potent carcinogenic potential including polycyclic aromatic hydrocarbons, nicotine and the nicotine-derived nitrosamines 4-(methylnitrosamino)-1-(3-pyrydyl)-1-butanone (NNK) and N-nitrosonornicotine (11). The most harmful and addictive component is nicotine (11). These carcinogens and their metabolites may induce the formation of DNA adducts which result in mutations of a number of key cancer suppressor genes, including retinoblastoma tumor suppressor protein (Rb), KRAS proto-oncogene, GTPase and tumor protein p53 (11) and eventually contributing to tumorigenesis in different ways. Accumulating evidences have suggested that nicotine not only contributes to tumorigenesis but may also increase the spread of cancer in the body (12-14).

It has been demonstrated that nicotine exerts its biological effects through nicotinic acetylcholine receptors (nAChRs) in human lung cancer cells (15). nAChRs are pentameric proteins composed of homologous subunits, which are encoded by a large multigene family (16,17). This receptor family was initially identified as notable regulators of the nervous system (18). In addition to their function in neuromuscular and motor autonomous transmission, nAChRs perform numerous central functions in almost all non-neuronal mammalian cells (18). The α7 subtype (α7nAChR), a subtype of nAChRs, is expressed in a variety of cells, including in endothelial cells, glial cells, brain radial glial cells, synovial cells and thymus cells, lymphocytes, bone marrow cells, monocytes, macrophages and microglia (19-21). Numerous studies have revealed that α7nAChR serves a notable function in the oncogenic process (22-24). In accordance with this notion, α7nAChR has been implicated in the proliferative, pro-angiogenic and pro-metastatic effects of nicotine in lung cancer types (24-27). Therefore, the α7nAChR-associated signaling networks in tumor cells may represent a novel target for the therapy of nicotine-associated lung cancer types.

The present review overviews evidence of previous studies to demonstrate the effects and molecular mechanisms of α7nAChR in lung cancer, and then describes the potential association of these signaling pathways with cancer-associated processes.

2. Epidemiology of lung cancer

Lung cancer is one of most deadly carcinoma types globally (28,29). Despite improvements in the diagnosis and treatment of this malignancy in previous years, the incidence and mortality rates of lung cancer are increasing. Based on Globocan 2012 estimates, lung cancer is the most commonly occuring cancer type among men in developed and developing countries and has exceeded breast cancer as the leading cause of cancer mortality amongst women in developed countries (30). In 2012, a total of ~1.8 million people were affected by this disease, and the estimated mortality rate was 1,098,700 and 491,200 for men and women, respectively (31). Amongst men, the highest lung cancer incidence rates were in Europe, Eastern Asia and Northern America, while the lowest incidence rates were in sub-Saharan Africa. Amongst women, the highest lung cancer rates were in Northern America, Northern and Western Europe, Australia/New Zealand and Eastern Asia (31). In China, lung cancer is the most commonly occurring cancer and the leading cause of cancer-associated mortality (32). Notably, this disease has one of the worst prognoses of all malignant tumor types and the overall 5-year survival rate is ~17.8% (33). Therefore, it is important to develop novel effective strategies in treatment of lung cancer.

Smoking is a key risk factor for lung cancer. The increase in lung cancer incidence globally parallels the rise of cigarette consumption (34). Particularly of note is that smoking is associated with 90% of SCLC and 60% of NSCLC cases and is responsible for ~80% of lung cancer mortality (35). A previous analysis revealed that passive smoking resulted in a higher risk of developing lung cancer compared with non-smokers (36). According to the U.S. Surgeon General, the risk of lung cancer in a non-smoker living with a smoker may be increased by 20-30% compared with a non-smoker living without a smoker (37). Notably, in countries where the tobacco epidemic has been established more recently, including in China, Indonesia and several countries in Africa, lung cancer rates are expected to continue to increase at least for the next few decades (38). Thus, apart from primary prevention programs including an effective tobacco-control policy, novel target molecules and the potential mechanisms of tobacco-associated lung cancer may attract more attention and should be further evaluated in future studies.

3. Expression of α7nAChR in lung cancer

Previous studies have revealed that nicotine-mediated tumor progression is initiated through the activation of nAChRs, specifically the α7 subunit (39-42). nAChRs belong to the superfamily of ligand-gated ion channels, including the excitatory $5HT_3$ receptor and the inhibitory receptors for glycine and γ-aminobutyric acid (43). To date, a number of nAChRs comprising various combinations of subunits have been identified (α1-α10, β1-β4, γ, δ and ε) (16,17). These receptors are activated by tumor cells contributing to the initiation of the non-adrenergic, non-cholinergic signaling, thereby promoting proliferation, angiogenesis and migration through autocrine and paracrine effects in lung cancer (44,45).

α7nAChR is expressed in several types of human lung cancer, including squamous cell lung cancer cells, lung adenocarcinoma and SCLC (11,46-49). Notably, the levels of α7nAChR expression are higher in squamous carcinoma compared with adenocarcinoma, particularly in smokers (50). In addition, there are different responses to cigarette smoking between women and men. α7nAChR expression is higher in male patients that smoked compared with female patients that smoked (51). Based on these observations, it has been proposed that α7nAChR upregulation in lung cancer cells may be involved in the nicotine-induced tumorigenic process (50,51). Future studies are required to explore the characteristics of α7nAChR which are emerging as a potential target for lung cancer therapy.

4. Roles and mechanisms of α7nAChR in lung cancer

Regulatory function of α7nAChR in lung cancer. Although nAChRs are widely expressed in non-neuronal and lung cancer cells, nicotine-mediated tumor progression is facilitated predominantly through α7nAChR (44,45). Consistent with this, α7nAChR levels have been revealed to be elevated in human squamous-cell lung cancer cells during sustained nicotine exposure (49). Similarly, the levels of α7nAChR in squamous cell carcinoma of lung tissues isolated from patients (who are active smokers) correlate with their smoking history (49). In addition, a previous study has revealed that α7nAChR levels were increased in mice that were administered nicotine (52), and nicotine-mediated effects on cell proliferation, invasion, migration and angiogenic tubule formation are abrogated in the presence of α7nAChR-specific inhibitors (53). Therefore, studying the role of α7nAChR and its underlying molecular mechanisms in lung cancer is clinically relevant.

A majority of mechanistic studies (27,47,49,51,53-69) focus on identifying the function of α7nAChR-mediated signaling

Table I. Regulatory function of α7nAChR in lung cancer.

Author, year	Targeted cell type(s)	Major outcome(s) associated with α7nAChR	(Refs.)
Zhang *et al*, 2016	NSCLCs	NSCLC cell invasion, migration and epithelial-mesenchymal transition were mediated by α7nAChR and MEK/ERK signaling pathway induced by nicotine	(27)
Dasgupta *et al*, 2006	NSCLCs	α7nAChR regulated the oncogenic process which depends on proliferation and survival-associated genes induced by nicotine	(47)
Brown *et al*, 2013	SCCLs	Upregulation of α7nAChRs accelerated tumor proliferation and progression through binding GATA4 or GATA6 stimulated by nicotine	(49)
Paleari *et al*, 2008	NSCLCs	α7nAChR promoted tumor cell growth by activating the Rb-Raf-1/phospho-ERK/phospho-$p90^{RSK}$ pathway	(51)
Medjber *et al*, 2015	NSCLCs	α7nAChR regulated cell growth and stimulated tumor invasion depending on the differentiation status of the tumor in NSCLCs	(53)
Al-Wadei *et al*, 2012	NSCLCs	α7nAChR promoted proliferation in nicotine-treated NSCLC cells by upregulating the stress neurotransmitter noradrenaline	(54)
Zovko *et al*, 2013	NSCLCs	APS8 inhibited cell growth and triggered the intrinsic apoptotic pathways	(55)
Paleari *et al*, 2009	NSCLCs (A549 cells)	α-CbT specifically inhibited the α7nAChR-mediated survival pathway	(56)
Grozio *et al*, 2008	NSCLCs (A549 cells)	α-CbT may reduce the tumor cell growth factors of nicotine	(57)
Sheppard *et al*, 2000	SCLCs	Activation of $Ca2^{+}$ influx contributed to the development of SCLCs by binding α7nAChR induced by NNK	(58)
Jull *et al*, 2001	SCLCs	NNK regulated the SCLCs growth by initiating the Raf-1/MAPK/c-myc kinase pathway i*n vitro*	(59)
Hung *et al*, 2009	CL1.0 lung cancer cells	NNK activated α7nAChR downstream signaling pathways of Akt and ERK	(60)
Zhong *et al*, 2015	NSCLCs	PGE2 increased the expression of α7nAChR by activating signals of JNK, PI3K and PKA through upregulating c-Jun	(61)
Sun *et al*, 2009	NSCLCs	Nicotine upregulated the expression of PPARβ/δ through α7nAChR-mediated activation of PI3K/mTOR signals and suppression of AP-2α protein expression and DNA binding activity in the PPARβ/δ gene promoter	(62)
Chernyavsky *et al*, 2015	SCCLs (SW900)	Activation of α7nAChR is associated with EGF and VEGF receptors in cell membrane	(63)
Brown *et al*, 2012	SCLCs	MG624 inhibited the angiogenesis of human SCLC tumor types followed by the suppression of nicotine-induced FGF2	(64)
Shen *et al*, 2012	Lung cancer cell lines (i.e. H1299, H82, H157 cells and H460 cells)	α-BTX blocked the tyrosine phosphorylation of c-Src, PKCι and FAK and prevented metastatic tumor types induced by NNK	(65)
Iskandar *et al*, 2016	Lung cancer cells	BCX restrains the migration and invasion of α7nAChR-positive lung cancer cells through the downregulation of α7nAChR/PI3K signaling	(66)
Zhang *et al*, 2017	NSCLCs (H1299)	Blocking α7nAChRs suppresses nicotine-induced H1299 cell proliferation are mediated through the de-phosphor ylation of the MEK signaling pathway in H1299 cells	(67)
Mucchietto *et al*, 2017	NSCLCs (A549 cells)	In A549 cells, α7 nAChR not only regulate nicotine-induced cell proliferation but also the activation of the Akt and ERK pathways	(68)

Table I. Continued.

Author, year	Targeted cell type(s)	Major outcome(s) associated with α7nAChR	(Refs.)
Yan *et al*, 2017	NSCLCs (A549 cells)	The methyllycaconitine citrate hydrate MLA and rL-RVG (the rabies virus glycoprotein) treatments significantly inhibited proliferation and migration and promoted apoptosis in the lung cancer cells	(69)

α7nAChR, α7 nicotinic acetylcholine receptor; NSCLC, non-small-cell lung carcinoma; SCCL, small cell carcinoma of the lung; SCLC, small-cell lung carcinoma; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinase; Rb, retinoblastoma tumor suppressor protein; Raf-1, RAF proto-oncogene serine/threonine-protein kinase; $p90^{RSK}$, MAPK-activated protein kinase-1; GATA, GATA binding protein; APS8, an analog of 3-alkylpyridinium polymers with a defined alkyl chain length and molecular size; α-CbT, α-cobratoxin; NNK, nicotine-derived nitrosamines 4-(methy lnitrosamino)-1-(3-pyrydyl)-1-butanone; MAPK, mitogen-activated protein kinase; c-myc, MYC proto-oncogene, BHLH transcription factor; Akt, protein kinase B; PGE2, prostaglandin E2; JNK, c-Jun N-terminal kinase; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PPAR, peroxisome proliferator-activated receptors; mTOR, mammalian target of rapamycin; AP-2α, transcription factor AP-2 α; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; MG624, an α7-nicotinic receptor antagonist; α-BTX, α-Bungarotoxin; MLA, an α7 nAChR antagonist; FGF2, fibroblast growth factor 2; c-Src, proto-oncogene tyrosine-protein kinase Src; PKCι, protein kinase Cι; FAK, focal adhesion kinase; BCX, β-cryptoxanthin.

in the regulation of the tumorigenic process including in proliferation, angiogenesis and metastasis in lung cancer (Table I). Notably, a number of α7nAChRs antagonists have been investigated to explore its influence on tumor progression (56,57,64-66,69). Provided that α7nAChR is a major genetic biomarker of nAChRs for lung cancer (70), strategies that target α7nAChR may be useful in the treatment of lung cancer for therapeutic purposes.

Function and mechanisms of α7nAChR on cell proliferation. At present, α7nAChR has been proposed to mediate nicotine-induced survival rate and proliferation in cancer cells *in vitro* and *in vivo* (22,70). It was revealed that proliferative signaling via α7nAChR required the scaffolding protein β-arrestin, while the ablation of β-arrestin or disruption of the Rb-RAF proto-oncogene serine/threonine-protein kinase (Raf-1) interaction blocked the nicotine-induced proliferation of NSCLCs (47). Furthermore, the α7nAChR-induced release of noradrenaline significantly stimulated NSCLC proliferation associated with the induction of phosphorylated (p)-extracellular signal-regulated kinases (ERK) and p-cAMP response element-binding protein signaling, suggesting that α7nAChR represents an attractive target for developing more effective intervention strategies for NSCLC (54). A previous study demonstrated that exposure to nicotine resulted in α7nAChRs upregulation in human squamous cell lung cancer via the Sp1 transcription factor/GATA binding protein pathway, which accelerates tumor proliferation and progression (49). However, several signals underlying α7nAChR-induced cell proliferation included the activation of $Ca2^{+}$ influx (58), Raf-1 (51,59), mitogen-activated protein kinase/ERK (27,51,59,60), c-Jun N-terminal kinase, phosphoinositide-3 kinase (PI3K)/protein kinase B (Akt), protein kinase A (PKA) pathway (60-62), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) receptors (63), and mitogen-activated protein kinase kinase (MEK)/ERK (67). In nicotine-induced lung cancer cells, Chernyavsky *et al* (63) revealed that the activation of cell membrane α7nAChR resulted in the association with EGF receptors, whereas activated mitochondrial α7nAChR physically associated with the intramitochondrial protein kinases PI3K and Src. Zhang *et al* (67) demonstrated that the blockade of α7nAChR specifically inhibited nicotine-stimulated tumor growth in NSCLC through the MEK/ERK signaling pathway. It has also been reported that α7nAChRs mediate the pro-proliferative effects of nicotine through activating Akt and ERK pathways, and blocking α7nAChRs eliminates nicotine-induced proliferation and signaling in A549 cells (68). These findings indicate that the expression of α7nAChR is associated with cellular survival rate and proliferation in lung cancer. A potential strategy may be to use α7nAChR as a biomarker to inhibit tumor proliferation and progression in lung cancer. Based on this information, α7nAChRs antagonists were revealed to attenuate the proliferative effects of nicotine in lung cancer (22). An analog of 3-alkylpyridinium polymers with a defined alkyl chain length and molecular size (APS8) may inhibit tumor may inhibit tumor growth and trigger the intrinsic apoptotic pathways in NSCLCs (55). Another study has confirmed that α7nAChRs antagonists including d-tubocurarine and α-cobratoxin (α-CbT) may reduce tumor cell growth factors stimulated by

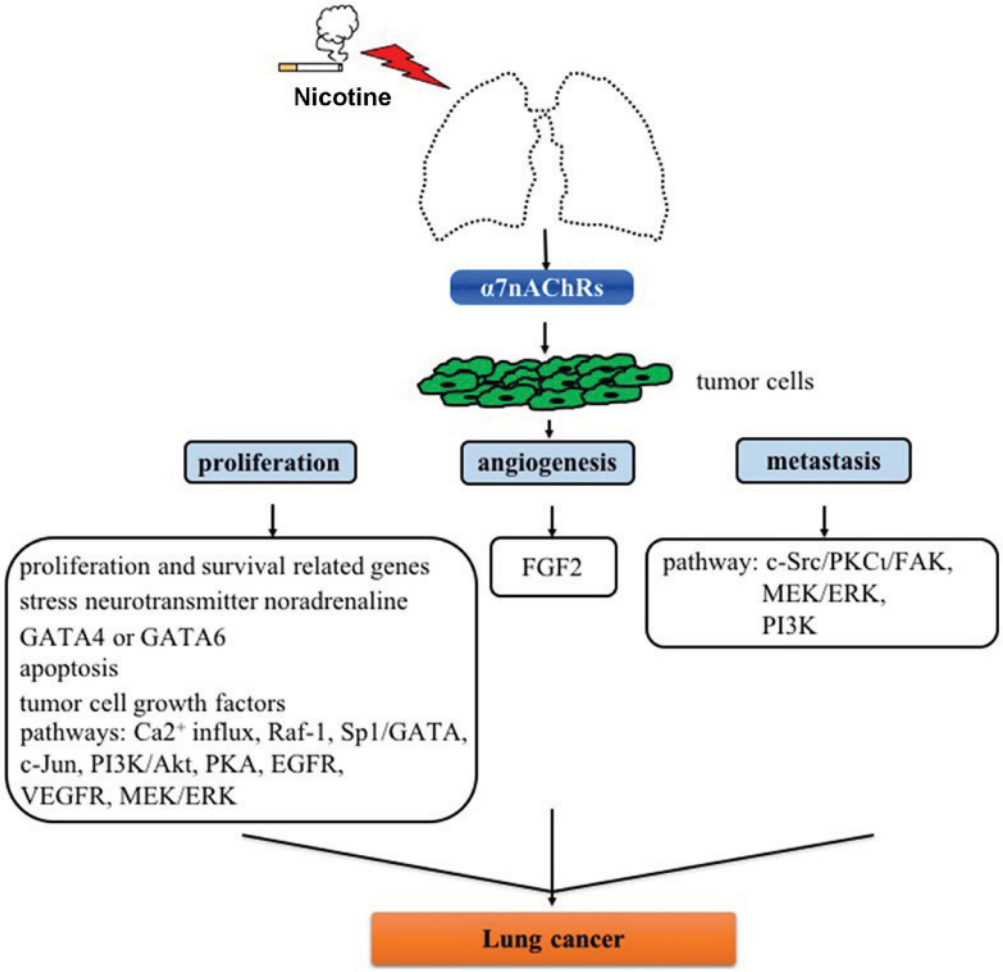

Figure 1. Role of α7nAChR on proliferation, angiogenesis and metastasis in lung cancer. α7nAChR, α7 nicotinic acetylcholine receptor; GATA, GATA binding protein; Raf-1, Raf-1 proto-oncogene, serine/threonine kinase; Sp1, Sp1 transcription factor; c-Jun N-terminal kinase; FGF2, fibroblast growth factor 2; c-Src, proto-oncogene tyrosine-protein kinase Src; PKCι, protein kinase Cι; FAK, focal adhesion kinase; MEK, mitogen-activated protein kinase kinase; ERK, extracellular signal-regulated kinases; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; EGFR, epidermal growth factor; VEGFR, vascular endothelial growth factor receptors.

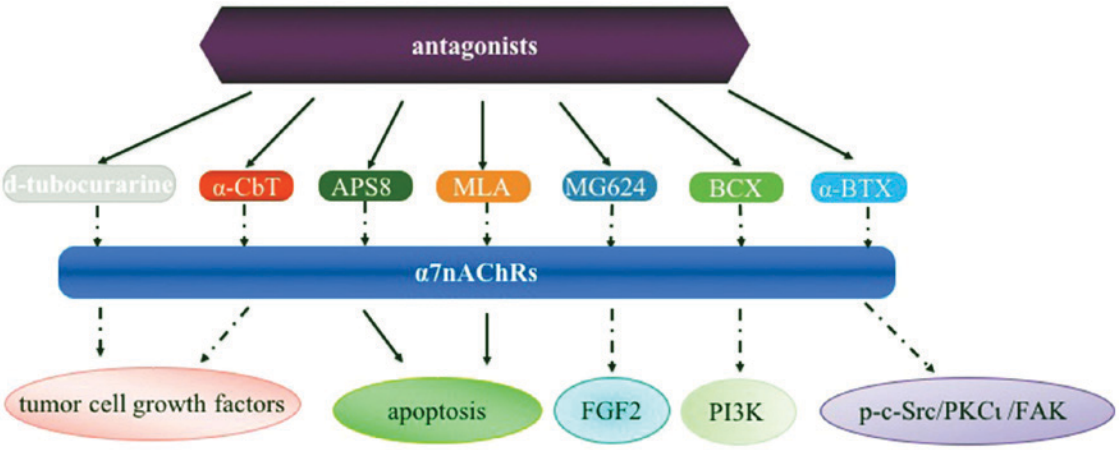

Figure 2. Overview of the α7nAChR antagonists in lung cancer. α7nAChR, α7 nicotinic acetylcholine receptor; α-CbT, α-cobratoxin; APS8, an analog of 3-alkylpyridinium polymers with a defined alkyl chain length and molecular size; MLA, an α7 nAChR antagonist; MG624, an α7-nicotinic receptor antagonist; BCX, β-cryptoxanthin; α-BTX, α-Bungarotoxin; FGF2, fibroblast growth factor 2; PI3K, phosphoinositide 3-kinase; p-c-Src, phosphorylated proto-oncogene tyrosine-protein kinase Src; PKCι, protein kinase Cι; FAK, focal adhesion kinase.

nicotine (56,57). Yan *et al* (69) revealed that methyl lycaconitine citrate hydrate (a α7nAChR antagonist) and rabies virus glycoprotein treatments significantly inhibited proliferation and promoted apoptosis in A549 lung adenocarcinoma cells.

Function and mechanisms of α7nAChR on angiogenesis. Angiogenesis is widely known as a typical characteristic in cancer to sustain tumor growth (71). Angiogenesis is necessary for primary tumor progression (72). Surprisingly, there is a limited study focusing on the angiogenic activity of α7nAChR in lung cancer. A previous study has demonstrated that the small-molecule antagonist for α7nAChR (MG624), inhibited angiogenesis effects in SCLCs followed by the suppression of nicotine-induced fibroblast growth factor 2 (64). Since α7nAChR upregulation by cancer cells stimulates tumor progression, it can be used in future studies to further explore its effects on angiogenesis.

Function and mechanisms of α7nAChR on metastasis. Metastasis is the major cause of mortality in cancer (73). The process of metastasis may be classically divided into a number of steps: Invasion of tumor cells into the surrounding tissues, penetration of vessels and migration toward distant sites of the body away from the primary sites (74). At present, several clinical studies in humans revealed an association between smoking and an increase in the metastasis of lung cancer (75-78). The α7nAChR is expressed in SCLC and NSCLC cells (24). Nicotine has a high affinity with α7nAChR in lung cancer cells (50). Thus, it would be useful to understand the mechanism of α7nAChR in metastasis in nicotine-associated lung cancer types (79). α7nAChR may regulate cell growth and stimulate tumor invasion depending on the differentiation status of the tumor in NSCLCs (53). The pro-proliferative activity of poorly-differentiated NSCLC was stimulated by nicotine, whereas it was suppressed in well-differentiated cells (53). Nicotine may also induce NSCLC cells invasion, migration and mesenchymal transition, which were mediated by α7nAChR involving the MEK/ERK signaling pathway (27). Meanwhile, the effects induced by nicotine may be suppressed by pharmacological intervention using α7nAChR selective antagonists or by genetic intervention using α7nAChR small interfering RNAs (55,68). α-bungarotoxin appeared to be one of the specific inhibitor for α7nAChR, which blocked metastatic tumors by NNK-induced tyrosine phosphorylation of proto-oncogene tyrosine-protein kinase Src, protein kinase Cι and focal adhesion kinase (65). In addition, β-cryptoxanthin treatment restrained the migration and invasion of α7nAChR-positive lung cancer cells through the downregulation of α7nAChR/PI3K signaling (66). All the aforementioned results suggest that α7nAChR enhances the metastasis of lung cancer cells, although the underlying molecular mechanisms require further investigation.

5. Conclusions

Despite efforts that have been reported focusing on α7nAChR as a molecular target in human diseases including lung cancer, a number of issues remain be addressed in future studies: i) Currently available evidence indicates that α7nAChR activation activates signaling pathways involved in the proliferation, angiogenesis and metastasis for developing lung cancer (Fig. 1), and thus it is crucial to analyze the difference of α7nAChR expression and underlying mechanisms in SCLC and NSCLC cells; ii) Little is known on the roles of these pathways in cell types including macrophages and other immune cells which are also very important in tumorigenesis; iii) α7nAChR expression is activated in the process of nicotine-mediated cancer; however, how α7nAChR antagonists (e.g., α-CbT treatment) are regulated in lung cancer is uclear (Fig. 2). Nevertheless, although there are several limitations for α7nAChR-based drug therapy for clinical use for lung cancer or other diseases, these potential mechanisms are inevitably the foundation of designing novel anticancer drugs in lung cancer.

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Authors' contributions

SCW and YH conceived and wrote the paper. YH reviewed and made final approval of the version to be published. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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