



Acetylcholine receptors: Key players in cancer development

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ABSTRACT

Acetylcholine (ACh) was first identified as a classic neuromodulator and transmit signals through two subgroups of receptors, namely muscarinic receptors (mAChRs) and nicotinic receptors (nAChRs). Apart from its well-established physiological role in central nervous system (CNS) and peripheral nervous system (PNS), autonomic nervous system and neuromuscular junction, the widely distributed expression of AChRs in different human organs suggests roles in other biological processes in addition to synaptic transmission. Accumulating evidence revealed that cancer cell processes such as proliferation, apoptosis, angiogenesis and even epithelial-mesenchymal transition (EMT) are mediated by overexpression of AChRs in different kinds of tumors. In breast cancer, $\alpha 7$ -nAChR and $\alpha 9$ -nAChR were reported to be oncogenic. On the other hand, research on the role of mAChRs in breast cancer tumorigenesis is limited and confined to M3 receptor only. Since AChRs distributed in both CNS and PNS even non-neuronal tissues, there is an urgent need for the development of subtype-specific AChR antagonist which inhibits cancer cell progression with minimal intervention on the normal acetylcholine-regulated system within human body.

1. Introduction

Acetylcholine receptors (AChRs) are neurotransmitter receptors that are classified into two major subtypes, ionotropic nicotinic acetylcholine receptors (nAChRs) and metabotropic muscarinic acetylcholine receptors (mAChRs). AChRs mediate neurotransmission in different aspects through endogenous neurotransmitter of parasympathetic nervous system, acetylcholine [1,2]. Neuronal acetylcholine is synthesized by choline acetyltransferase (ChAT) from choline and acetyl-CoA, packaged by vesicular acetylcholine transporter (VAChT) as vesicles and released by exocytosis for neurotransmission by preganglionic neurons [3]. Numerous studies have demonstrated the production of acetylcholine and expression of AChRs in non-neuronal cells regulate important cell functions such as cell proliferation, migration and differentiation [3–8]. Breast cancer development, as well as other cancers, is a multifactorial process involving different etiologies and genetic risks. Overwhelming evidence has suggested that acetylcholine and AChRs are important regulators in tumorigenesis [3]. In this review, we will focus on the relationship between AChRs and different kinds of cancers, with special emphasis on breast cancer.

2. Nicotinic acetylcholine receptors (nAChRs)

nAChRs are fast ionotropic cationic nicotinic receptors that mediate fast synaptic transmission [1,9]. nAChRs are pentameric transmembrane protein complexes formed by five receptor subunits (Table 1). Seventeen subunits of nAChRs have been characterized, in which 12 subunits are known neuronal-type nAChRs ($\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$) and 5 subunits are muscle-type nAChRs ($\alpha 1$, $\beta 1$, δ , γ and ϵ) [8]. Among all nAChR subunits, $\alpha 8$ is not expressed in human cells [10]. In neurons, functional neuronal homopentamers can be formed by $\alpha 7$, $\alpha 8$ and $\alpha 9$ only while heteropentamers are formed by combination of α subunits and β subunits [3]. In muscle, functional heteropentamer consisted of two α subunits and one of each β , γ and δ subunits [7–9]. Binding of nAChRs to acetylcholine or nAChRs agonists leads to membrane depolarization and opening of voltage-gated ion channels, resulting influx and efflux of ions [10,11]. Additional calcium influx triggers the release of cell-type specific signaling molecules, such as neurotransmitters, growth and angiogenic factors through exocytosis [11]. nAChR mediates normal physiological responses, such as inflammation and pain, the dysregulation of nAChRs are associated with neurological disorders such as Alzheimer's disease and Parkinson's disease [12]. Therefore, therapeutic potential of using nAChR agonists, for example, nicotine, as

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Table 1
Acetylcholine receptor (AChR) subtypes.

Subtypes	Family	Structure	Location	Subunits	Agonists	Antagonists
Nicotinic	Ionotropic (ligand-gated ion channel receptors)	Pentameric transmembrane proteins	Neurons Ganglia Interneurons Motor endplate	Neuronal: $\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$ ($\alpha 8$ not found in human) Muscle-type: $\alpha 1$, $\beta 1$, δ , γ (developmental muscle), and ϵ (mature muscle)	Nicotine Succinylcholine	α -Bungarotoxin
Muscarinic	Metabotropic (G protein-coupled receptors)	Seven-transmembrane proteins	Neurons Heart Smooth muscle fibers Glands	M1-M5	Muscarine	Atropine

potent anti-inflammatory agents and analgesics have been investigated [7,12]. nAChRs were believed to be expressed only in nervous system for decades until the discovery of its expression in lung cancer cells [11]. Recent studies have demonstrated different expression patterns of nAChRs in non-neuronal cells, including endothelial cells and skin keratinocytes, as well as cancer cells [5,9,13]. Epibatidine, an alkaloid extracted from the skin of Ecuadorian tree frogs, and nicotine, a major addictive from cigarette smoke, are naturally occurring nAChR agonists [7,10]. In addition, synthesis and secretion of acetylcholine by cancer cells creates a cholinergic autocrine-loop to stimulate cancer cell proliferation [5,14,15].

2.1. The role of nicotine and its derivative in cancer progression modulated by nAChRs

Cigarette smoke is a complex mixture that contains approximately 5000 chemical components, including more than 60 carcinogens [10]. Among these components, nicotine is the major addictive in cigarette smoke. Although nicotine is not considered as carcinogenic, it is tumor-promoting by inducing cell proliferation through the binding of nAChRs [11]. The proliferative effect of nicotine on small cell lung cancer (SCLC) and non-small cell lung cancers (NSCLC) has been investigated extensively. Nicotine has also been reported to promote various types of cancers such as breast, head and neck, gastric, liver, and cervical cancers [10]. Besides nicotine, numerous studies have reported the carcinogenic effects of nicotine-derived nitrosamines such as cotinine, N'-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to nAChRs in lung cancers [10]. The binding affinity of nicotine-derived nitrosamines to nAChRs is higher than nicotine [11,16]. Among all nAChRs, $\alpha 7$ -nAChR is the major nAChR that expressed in lung, colon, and bladder cancer cells, while $\alpha 7$ -nAChR and $\alpha 9$ -nAChR are highly expressed in breast cancer cells [17,18]. In particular, $\alpha 7$ -nAChR, $\alpha 9$ -nAChR and heteromeric nAChRs containing $\alpha 9$ and $\alpha 10$ subunits are selective for calcium ions [10]. Influx of calcium ions upon stimulation of nAChRs triggers the release of neurotransmitters and autocrine growth factors that induce cancer cell proliferation and inhibit apoptosis [11].

2.2. Autocrine/paracrine acetylcholine-mediated tumorigenesis by AChRs

Besides nitrosamine-mediated tumorigenesis, several studies have demonstrated that cancer cells are able to synthesize acetylcholine to promote tumorigenesis through AChRs in the absence of nicotine or AChR agonists [4,5,14,15,19]. Release of acetylcholine was first demonstrated in SCLC and expression of ChAT was found in six SCLC cell lines (H69, H82, H345, H378, H417 and H592). Synthesis and secretion of acetylcholine were inhibited by VAcHT inhibitor, vesamicol, at a dose-dependent manner in SCLC [15]. Similar findings were also reported in colon and gastric cancers. For instance, MKN45 and BGC823 gastric cancer cells produced acetylcholine, which promoted cell growth in a dose-dependent manner [4]. In HGC27 and MGC803 gastric cancer cells, acetylcholine stimulated cell proliferation and was

abolished by blockade of AChR [19]. Similarly, H508 and Caco-2 colon cancer cells released the self-produced acetylcholine which subsequently promoted cell viability in an autocrine manner [5]. Expression of ChAT was reported in gastric cancer cell lines (MKN28, MKN45, BGC823, MGC803 and SGC7901) and the secretion of acetylcholine by cancer cells was greatly impeded after knockdown of ChAT [4]. Taken together, this evidence suggests the importance of acetylcholine secretion in cancer cells in AChRs-mediated cancer progression.

2.3. Regulatory pathways of nAChRs in cancers

Activation of nAChRs regulates numerous signaling cascades depends on nAChR subtypes in a cell-type specific manner. For instance, activation of $\alpha 7$ -nAChR by nicotine activated Ras/ERK/MAPK and JAK2/STAT-PI3K pathways, leading to cancer cell proliferation and migration in lung cancer cells [10,11]. Furthermore, the release of stress neurotransmitters such as epinephrine and norepinephrine from sympathetic nervous system will also be triggered, results in activation of β -adrenergic receptors (β -AR) [16]. β -AR is G-protein coupled receptors and its involvement in cancer progression is well-documented. Other than function as an AChR agonist, nicotine has also been reported to bind to β -ARs [20,21]. Activated β -ARs stimulate the release of arachidonic acid (AA), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) via adenylyl cyclase/cAMP/PKA/CREB pathway during cancer progression and promote angiogenesis [16]. Nicotine and NNK have also reported to stimulate the expression of COX-2 in lung and gastric cancers [22,23]. Upregulation of COX-2 stimulated the expression of VEGFR and modulated activities of matrix metalloproteases, leading to tumor progression through β -AR [20]. Recent studies have revealed the role of insulin-like growth factors (IGFs) -mediated lung cancer tumorigenesis through nAChR [24,25]. NNK promoted secretion of IGF1 and IGF2 and overexpression of phosphorylated insulin-like growth factor receptor 1 (IGF-R1) were higher in lung tumor tissues from smokers when compared with non-smokers [24,25].

2.4. Involvement of nAChRs in breast cancer

As mentioned in previous sections, $\alpha 7$ -nAChR and $\alpha 9$ -nAChR are the major nAChRs present in breast cancer cells. Studies regarding the involvement of nAChRs in breast cancer are listed in Table 2. In consistent with studies in other cancers, activation of $\alpha 7$ -nAChR leads to activation of ERK/MAPK and JAK2/PI3K signaling cascades in breast cancer cells [26–28]. In terms of signaling cascades regulated by $\alpha 9$ -nAChR, nicotine induced overexpression of cyclin D3 in breast cancer [29]. The action of $\alpha 9$ -nAChR was further investigated by inducing its expression using doxycycline in MCF-10A and in breast cancer xenograft models [17,29]. Nicotine- or NNK-transformed MCF-10A and breast cancer cell lines increased cell proliferation *in vitro* and *in vivo*. Another study demonstrated that nicotine mediated STAT3/TWIST1 pathway in MCF-7 through $\alpha 9$ -nAChR [30]. Inhibition of breast cell proliferation was observed after knockdown of $\alpha 9$ -nAChR *in vitro* and *in*

Table 2
Summary of 10 published studies of nAChRs (Breast cancer only).

Receptor subtypes	Agonist(s)	Subtypes Cancer type/Cell line	Pathways	Type of studies (<i>in vitro</i> , <i>in vivo</i> etc)	Cohort size (if involved human) subjects	Type of human specimens	Reference
α9-nAChR	Nicotine	MCF-10A MDA-MB-231	Cyclin D3	<i>in vitro</i> and <i>in vivo</i>	/	/	[29]
α9-nAChR	Nicotine	MCF-10A MDA-MB-231 MCF-7	/	<i>in vitro</i> and <i>in vivo</i>	276	Tumor tissues	[17]
α9-nAChR	Nicotine	MCF-7	STAT3/ TWIST1	<i>in vitro</i>	22	Tumor tissues	[30]
α7-nAChR	NNK	AU565 ZR-75-1 HCC1395 MDA-MB-231 Human Mammary Epithelial Cells (HMEC)	ERK1/2/MAPK	<i>in vitro</i>	/	/	[26]
α7-nAChR	Nicotine	MCF-7	PKC/NOTCH	<i>in vitro</i>	/	/	[118]
α9-nAChR	/	MCF-7	ER	<i>in vitro</i>	339	Tumor tissues	[33]
Non-selective nAChR	Nicotine	MCF-10A MDA-MB-231	EGFR/ERK1/2 AKT/BCL-2	<i>in vitro</i>	/	/	[28]
α7-nAChR and α9-nAChR	NNK	MCF-7 MCF10A	ERK1/2	<i>In vitro</i>	/	/	[27]
α9-nAChR	/	MDA-MB-231 1LM and 2LM ^b Phoenix-ECO ^a	/	<i>In vitro</i> and <i>in vivo</i>	/	/	[31]
α9-nAChR	Nicotine	AU565 BT474 SKBR3 ZR75 BT483 T47D MDA-MB-231, MCF-7 MDA-MB-453 HS578T HBL100 MCF- 10A	PPM1F	<i>In vitro</i> and <i>in vivo</i>	167	Tumor tissues	[32]

Abbreviation: MAPK – Mitogen-activated protein kinases; ERK – Extracellular-signal-regulated kinases; ER – Estrogen receptor; PPM1F –protein phosphatase, Mg2+ /Mn2+ dependent 1F

NNK – 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone.

^a Human kidney epithelial cells.

^b MDA-MB-231-derived first generation and second generation spontaneous lung metastatic (1LM and 2LM) cells.

Table 3
Summary of muscarinic receptors and their reported literature with cancers.

Receptor subtypes	Agonist(s)	Antagonist(s)	Organs distribution	Signal transduction pathway	Reported literature with cancers	References
M1R	AC-260584, TBPB	Pirenzepine	GI epithelium, salivary glands	phospholipase C activation	prostate cancer	[82,84]
M2R	Arecaidine propargyl ester tosylate	THRX-160209, Himbacine	heart, smooth muscle, CNS	adenylyl cyclase inhibition	bladder cancer glioblastoma non-small cell lung cancer	[86] [85] [119]
M3R	Cevimeline	4-DAMP, Darifenacin	salivary glands, smooth muscle, CNS	phospholipase C activation	breast cancer colon cancer gastric cancer lung cancer melanoma ovarian cancer	[102–107] [92,94] [4] [88,89] [97–99] [101]
M4R	LY2033298	AFDX-384	CNS, lung, uterus	adenylyl cyclase inhibition	/	/
M5R	VU-0238429	ML381	CNS (substantia nigra), eye	phospholipase C activation	/	/

Abbreviation: GI – gastrointestinal; CNS – central nervous system; AC-260584 – (4-[3-(4-butylpiperidin-1-yl)-propyl]-7- fluoro-4H-benzo [1,4]oxazin-3-one; TBPB – [1-(1-(2-methylbenzyl)-1,4-bipiperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one]; 4-DAMP – (1,1-dimethyl-4-diphenylacetoxypiperidinium iodide); ML381 – 5-[(3-Acetylphenoxy)methyl]-N-methyl-N-[(1S)-1-pyridin-2-ylethyl]-1,2-oxazole-3-carboxamide fumarate; LY2033298 – 3-Amino-5-chloro-N-cyclopropyl-6-methoxy-4-methyl-thieno [2,3-b]pyridine-2-carboxamide; VU-0238429 – 1-(4-Methoxybenzyl)-5-trifluoromethoxyisatin.

in vivo [17]. Recent studies by same research group demonstrated the importance of α9-nAChR in mediating TNBC lung metastasis and the potential downstream molecular targets of α9-nAChR [31,32]. Induction of α9-nAChR by nicotine significantly upregulated expression of PPM1F, a protein phosphatase, which suppressed the activity of p53 to promote breast cancer proliferation [32].

Notably, there were only 4 studies reported the involvement of human subjects with different sample sizes [17,30,32,33]. Compared with adjacent normal tissues, α9-nAChR was overexpressed in tumor tissues and its expression was correlated with advanced stage breast cancer [17]. Interestingly, overexpression of α9-nAChR was reported to upregulate the expression of estrogen receptor (ER), and yet high

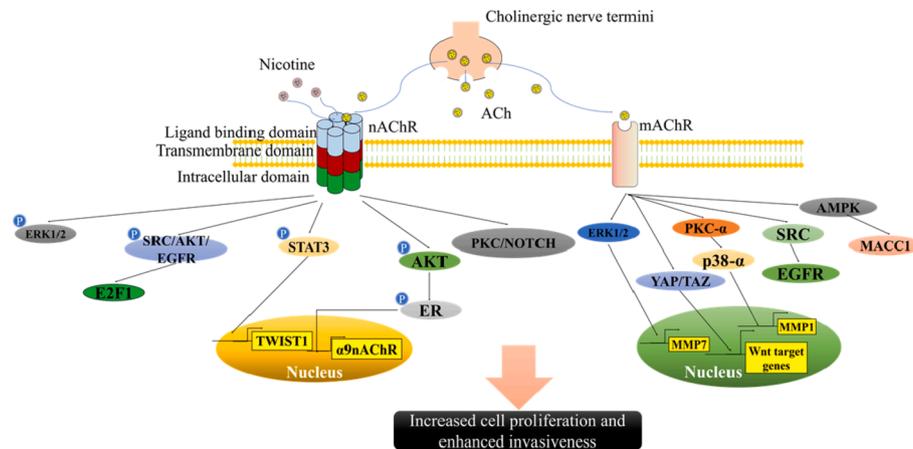


Fig. 1. The dysregulated signaling pathways controlled by AChRs in cancers.

Abbreviation: ERK – Extracellular regulated kinases; EGFR – epidermal growth factor receptor; ER – estrogen receptor; PKC – Protein kinase C; MMP – Matrix metalloproteinases; MACC – MET Transcriptional Regulator.

expression level of $\alpha 9$ -nAChR was associated with poor 5-year disease-specific survival [33]. Both $\alpha 9$ -nAChR and its downstream target, *PPM1F*, were upregulated significantly in smoker when compared with non-smoker [30,32]. Collectively, these studies provide evidence which suggests the critical role of nAChRs in breast cancer tumorigenesis.

3. Muscarinic acetylcholine receptors (mAChRs)

Another class of AChR is mAChR, it belongs to a class of heptahelel G protein-coupled receptors and consists of five distinct subtypes (M1-M5), in which they trigger second messenger upon activation (Table 1). Individual receptors are preferentially coupled to distinct G proteins. Signals transmitted through M1, M3 and M5 receptors activate phospholipase C, while inhibits adenylyl cyclase by M2 and M4 receptors [34,35]. mAChRs are capable to activate multiple signaling pathways in addition to the canonical ones. For example, M1, M3, and M5 receptors stimulate phospholipase A2, phospholipase D, tyrosine kinase, as well as calcium channels. M2 and M4 receptors also have the ability to activate phospholipase A2 as a second messenger [36].

3.1. Physiological functions of mAChRs

M1 receptor (M1R) is located at the ganglion in postganglionic nerve that mediates slow excitatory postsynaptic potential. High expression of M2R in heart signifies the importance in myocyte contraction and heart rate control [37–39]. Under normal condition, vagal parasympathetic neurons secrete acetylcholine to reduce heart rate via M2R [35,39]. Also, M2Rs are expressed in the CNS, such as spinal cord thalamus, at a much lower level than in the heart where they control the release of acetylcholine [40–42]. In addition, M2R was co-expressed with M3R in smooth muscle controlling contractile response [37]. Other processes such as thermoregulation, cognitive functions, behavioural flexibility and memory are at least partially regulated by M2R [43,44]. Alzheimer's and Parkinson's diseases are the two common neurological disorders which were found to be associated with loss of M2R expression in cerebral cortex [45,46].

Having a similar function to M1R, M3R is coupled to G proteins of class G_q . The function of this receptor includes cellular responses, such as preferentially activation of phospholipase C, phosphoinositide breakdown and inhibition of calcium-regulated potassium channels [47–49]. M3R mediates acetylcholine in CNS and PNS with various fundamental physiological functions. However, peripheral M3Rs are critical in mediating actions of acetylcholine on smooth muscle and glandular tissues [35,50,51]. On the other hand, M4R belongs to a larger family of G protein-coupled receptors which coupled to G_i/o

heterotrimeric proteins [52], and it is primarily located in the CNS [53–55]. M4R activation through adenylyl cyclase inhibition, phosphoinositide degeneration and potassium channel regulation acts as inhibitory autoreceptor for acetylcholine [56,57]. M4Rs are abundantly expressed in striatum [58] and activation of M4R inhibited dopamine-induced locomotor stimulation [57] while the M4R-deficient mice increased locomotor stimulation in the presence of dopamine agonists when compared with the M4R wild-type group [59,60]. Moreover, M4Rs are preferentially co-expressed with dopamine D1 receptors in striatonigral projection neurons within striatum [61,62]. Therefore, dysregulation of M4R will cause different kinds of mental disorders such as schizophrenia, and neurodegenerative disorders, for example, Parkinson's and Alzheimer's diseases [63,64].

The last muscarinic subtype, M5 receptor (M5R), belongs to the G protein-coupled receptor superfamily of integral membrane proteins and coupled to the G_q protein [52]. Activation of M5R results in phospholipid turnover and alteration in calcium concentration within the cells [65]. M5R expressed on dopamine neuron terminals in the substantia nigra and the ventral tegmental area (VTA) which facilitates the release of dopamine and glutamate from midbrain projections [66]. It is the exclusive muscarinic receptor that located in the VTA of the midbrain [53,66]. The exclusive expression of M5R in VTA implied its important role in reward system since VTA is involved in the brain's reward circuit [67]. Another important function of M5R is the regulatory effect on blood flow. Its expression present in various peripheral and cerebral blood vessels [68–70], activation of M5R causes relaxation of cerebral vascular tone [71]. Evidence supported that the ability of acetylcholine-mediated cerebral vessel dilation was lost in M5R-deficient mice when compared with their littermates [71]. Another study indicated that cholinergic neurons control stem cell proliferation which was due to the involvement of M5R [72].

3.2. Association of mAChRs with cancers

Since mAChRs are widely expressed in different locations such as CNS [73], gastrointestinal tract [74], urinary bladder [75,76], heart [77], lung [78], eye [73], vessels [71,79] and smooth muscle [80] with numerous physiological functions, the outcomes of alteration in specific muscarinic receptors are difficult to determine. Both intercellular and intracellular signalings are critical for neoplastic transformation to sustain cell proliferation. Despite the long discovery of mAChRs in normal human physiology, little attention has been given to the relationship between mAChRs and cancer development. Until recent years, there is growing evidence demonstrated that dysregulation of mAChRs in various kinds of human malignancies including breast,

colon, prostate and brain [2]. Different subtypes of mAChRs in relation with cancers were summarized in Table 3.

In prostate cancer, there is evidence support a link between cancer progression and cholinergic nerves stimulation [81]. M1R was identified to express over the cell membrane and cytoplasm in prostate cancer cells [82] and involved in the regulation of cancer metastasis [83]. Other study demonstrated the contribution of M1R on cancer cell migration and invasion through hedgehog signaling in prostate cancer [84]. In human glioblastoma, M2R agonist (arecaidine) inhibited cell proliferation and survival in cancer stem cells [85]. Recent study demonstrated that inhibition on cell viability was directly dependent on M2R activation but not M1R or M3R in urothelial bladder cancer cells [86]. Moreover, M3R has been demonstrated to induce various signaling pathways associated with cellular growth, invasion, migration, survival, inflammatory response and angiogenesis in numerous types of cancer cells such as breast, ovarian and brain cancers [87]. In addition, it is reported that M3R expression is significantly correlated with lung cancer metastasis and poor prognosis, while M3R specific antagonist (R2HBJJ) inhibited lung cancer cell proliferation [88,89]. Emerging evidence support that mAChRs signaling plays a critical role in growth and progression of colon cancer [90]. EGFR is commonly overexpressed in colon cancer tissues [91], M3R and EGFR are frequently co-expressed, suggesting a functional interaction with M3R in regulating colon cancer progression [92,93]. Further study confirmed that cholinergic ligand binding with M3R results in transactivation of EGFR, thus promoted cell proliferation [92]. Also, acetylcholine-induced selective matrix metalloproteinase genes through M3R which stimulated colon cancer cell growth [94]. Similarly, acetylcholine stimulated M3R to activate EGFR signaling and induced phosphorylation of ERK1/2 and AKT, whereas blocking EGFR inhibited acetylcholine-induced gastric cancer proliferation [4]. Another study identified that M3R promoted gastric tumorigenesis through Wnt and YAP pathways [95]. Other signaling pathway involving M3R/AMPK/MACC1 promoted cancer cell invasion and migration, suggested a potential underlying mechanism of gastric cancer growth and metastasis [96]. A number of studies have revealed a higher expression in melanomas and metastatic lesions [97–99]. Although expression of M3R and other muscarinic receptor subtypes has been characterized in different pancreatic cancer cell lines, functional roles in carcinogenesis have not been fully investigated [100]. In ovarian cancer, majority of tumor tissues expressed M3R, and the expression was associated with poor survival [101]. In breast cancer, the expression pattern of M3R is not well studied and characterized. Previous studies demonstrated that M3R stimulation promoted cell viability in breast cancer cells [102–104], which involved ERK1/2 activation [103]. Interestingly, M3R autoantibodies were detected in the circulation of breast cancer patients, and the stimulation of cell proliferation and migration is likely M3R-dependent [103,105–107]. In addition, M3R contributed to the cytotoxicity of chemotherapeutic drugs by modulating nitric oxide synthase and arginase activities [108]. To date, there is limited report on the relationship between M4/5R and cancer development. Therefore, the roles of these two receptors in tumors warrant further investigation. The possible signaling cascades in cancers regulated by AChRs are summarized in Fig. 1.

4. Genetic association of AChRs and cancer risk

Besides functional preclinical studies, large-scale genome-wide association studies (GWAS) and meta-analysis reported the association between lung cancer risk and single nucleotide polymorphisms (SNPs) in genes encoded for nAChRs [109–112]. A susceptibility locus at chromosome region 15q25 encoded for three subunits of nAChRs, namely, *CHRNA3*, *CNRNA5*, and *CHRNA4*, reported the association with risk of lung cancer in six independent populations [110]. Similar association between lung cancer risk and SNPs in *CHRNA3* and *CHRNA5* were also observed in two independent populations [109].

Candidate gene approach revealed the association between SNPs in nAChRs and increased susceptibility risk in esophageal, gastric and cervical cancers [113–115]. However, GWAS of susceptibility loci on genes encoded for nAChRs in breast cancers has not been explored. On the other hand, study on the genetic association between mAChRs and cancers is limited. There is a significant contribution of *CHRM3* gene in bladder cancer etiology among a Chinese Han population [116]. Another pathway analysis of GWAS data revealed *CHRM5* may be one of the major contributing genes in pancreatic cancer development [117]. Large cohort study is needed to confirm the genetic correlation between AChRs and cancers. Better understanding in this field would provide insights into the risk assessment and therapeutic alternatives for cancer patients.

5. Conclusions and future challenges

Since the discovery of nAChRs and mAChRs, there are tremendous studies on the biological functions, pharmacology and structures of these receptors. A number of intensive investigations have shed lights on the complicated network under the acetylcholine-regulated system in the past decades. As an integral part of normal physiological functions widely distributed in different organs, dysregulation of AChRs was shown to cause various diseases. Due to the wide range of regulatory pathways, AChRs are of great importance as therapeutic targets in treating different disorders. In recent years, the novel discoveries of AChRs on cancer development have drawn attention into their potentials in clinical application. Enormous research findings provided solid link between carcinogenesis and AChRs (nAChRs and mAChRs), and inhibition of cancer cells by AChR-specific blockers *in vitro*. However, AChR antagonists exhibit adverse effects on all the cells that express cholinergic receptors. In this regard, further investigation is needed to develop AChR-targeted therapies, without interrupting the normal physiological functions.

Potential competing interests

All authors declare that they have no competing financial interests.

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