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Review

Nicotinic Acetylcholine Receptors as Potential Tumor Biomarkers in Genitourinary Cancers: a Review Study

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Abstract

The genitourinary tissues express the different subtypes of nicotinic acetylcholine receptors (nAChRs), which are involved in many physiologic and pathologic processes. New studies have indicated the significant role of nAChRs in multiple tumor-related properties in different types of malignancies. Genitourinary cancers (GUCs) represent a heterogeneous population of cancers, in both histology and approach to treatment. nAChRs are functionally expressed by a variety of immune cells, tumor cells, and tumor-associated cells in the microenvironment of GUCs. In the current review study, publications until May 2021 were included in the literature review to summarize the potential effects and clinical and experimental significance of nAChRs in GUCs pathogenesis. The results yielded substantial and some paradoxical evidence regard the role of different subtypes of nAChRs as potential regulators and predictive biomarkers for GUCs. The accumulated evidence demonstrated that nAChRs levels were increased in the GUCs samples, which provides clinically relevant information on utilizing nAChRs as a new biomarker to improve the prognosis of these cancers. Also, activation or blockade of these receptors may lead to different downstream signaling pathways and cause diverse effects. Regarding the significant global burden of GUCs, evaluation of these receptors and delineating their molecular mechanisms could enrich our understanding of the biology of GUCs and may have new opportunities for clinical impacts.

Introduction

The genitourinary system is the number of organs related to the reproductive and urinary systems. Genitourinary cancers (GUCs) include a heterogeneous group of cancers that start in these organs and affect their function.[1] Among these cancers, kidney and bladder cancers in both sex, along with prostate, testis, and penile cancers in men and cervical, ovarian, and uterine cancers in females, are the most prevalent types of GUCs.[2] Along with some other unspecified GUCs, these cancers represent 25% of all solid tumors, and each of them is unique, with different signs and symptoms.[3] GUCs are highly divergent in terms of molecular pathology and prognosis, ranging from excellent outcomes for patients with testicular cancer to metastatic clear cell renal cell carcinoma, which is associated with poor outcomes.[2] In the early phases, GUCs can be successfully treated by surgical, pharmacological, and radiotherapy management but in the advanced stages, these treatment options have a limited or palliative effect.[4] Current methods for diagnosis and monitoring of GUCs are often invasive and have limited sensitivity and specificity.[5,6] Considering the high morbidity and mortality of the patients from GUCs each year, there remains an urgent and unmet need for the development of new tumor biomarkers and novel therapeutics.[7] Due to the lack of sensitive predictive biomarkers, the large number of GUCs patients are mostly detected at an advanced stage, and the 5-year survival rate remains far from satisfactory. Recently, researchers have been devoting themselves to identifying the novel tumor biomarkers associated with GUCs screening, diagnosis, prognosis, and evaluation of therapeutic efficacy to improve their survival rate. As the incidence of these cancers is on the rise, it is clinically urgent and necessary to explore reliable biomarkers which can predict the prognosis of genitourinary system cancers. Smoking is a well-known modifiable risk factor in the initiation and progression of many cancers, including GUCs[8] (Figure 1). Nicotine is the main component in cigarette smoke and binds to nicotinic acetylcholine receptors (nAChRs) and mediates its biological effects via

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the activation of these receptors. nAChRs are pentameric proteins, made up of five subunit ion channels, and are permeable to Na^+, K^+, or Ca^{+2} channels. nAChRs can form heteropentameric receptors containing α- and β-subunits, as well the subunits α7 and α9 can arrange as homopentameric cation channels[9] (Figure 1).

Nicotinic receptors express by many different types of genitourinary cells such as kidney,[10] bladder,[11] prostate,[12] testicular,[13] penile,[14] cervical,[15] ovarian,[16] uterine,[17] as well in their cancerous cells. Among the different subunits of nAChR, previous reports indicated that the nAChRs are associated with different genitourinary cancers-related properties including CSC renewal, proliferation, metastasis, angiogenesis, and suppression of apoptosis (Figure 2). Disrupted signaling via nAChR is associated with GUCs, but the exact underlying causes are not known.

In this study, PubMed, Google Scholar, Scopus, and Web of Science databases were searched using the terms "nicotinic receptors", "smoking", "nicotine", "tumor biomarker", "kidney cancer", "bladder cancer", "prostate cancer", "testicular cancer", "penile cancer", "cervical cancer", "ovarian cancer", "uterine cancer", and their synonyms and combinations. The diverse and paradoxical roles of nAChR and the principal controversies relating to the effects of nAChR on GUCs are in our focus in this review article. Nowadays, tobacco smoking is the cause of ~5–6 million deaths per year. Nicotine is the addictive component of tobacco acting on nAChR. It is hoped that identifying the role of nAChR in GUCs improved clinical outcomes of these cancers and created new treatment plans for GUC suffered patients. The present review study provides a rationale to develop novel nAChR-based diagnostic and therapeutic approaches to suppress the development progression of these types of malignancies.

Nicotine and its receptors in the cancer context

Nicotine is a lipophilic chiral alkaloid which naturally synthesized by a family of plants, most predominantly present in tobacco leaves.[18] This substance, first discovered by German chemists Poslett and Riemann in the tobacco plant, and its role in muscle tissue activation was discovered by Claude Bernard in 1852, which is now used for recreational and medical purposes.[19,20] Nicotine is generally present in both -S and -R isoforms that S isoform is more in nature. These two isoforms at first have a similar content of impurities because the nicotine from plants is used to produce them.[21] Nicotine is non-ionized in alkaline pH and is easily absorbed from the epithelium of the lungs, oral mucosa, nose, and skin.[22] The effects of smoking and the constituents of tobacco were investigated in numerous studies.[23] Today, nicotine is also used to treat addiction. Nicotine also has analgesic properties and reduces nicotine levels in smokers, leading to reduced morphine use.[24,25] Clinical data show that smoking improves cancer growth and resistance to treatment in cancer patients.[26–31] Tobacco use has been shown to cause 20% of cancer deaths worldwide.[32] Cigarettes have more than 60 carcinogenic molecules, including benzo [pyrrh] (BaP), multi-ring aromatic hydrocarbons, nicotine, and nitrosamines that increase the risk of lung cancer, stomach, liver, pancreas, transient cellular cancer of the bladder, and kidney cancer.[33–35]

Nicotine exerts its effects by binding to special pentameric transmembrane receptors named nAChRs. In the vertebrates, the nAChRs consist of 17 known subunits, which are 10α (α1-α10), 4β (β1-β4), γ, δ, and ε, which can assemble to form a diverse family of nAChR subtypes.[36,37] These receptors are among the ligand-activated ion channels that are permeable to various cations, including Na^+, K^+, or Ca^{+2}.[38] Unlike acetylcholine, nicotinic metabolism is not broken down by acetylcholinesterase and is initiated in the liver by the cytochrome P450 enzymes CYP2A6 and CYP2B6, and 70% of the nicotine absorbed from the gastrointestinal tract is converted to cotinine.[19,39] With a greater tendency to take acetylcholine receptors than acetylcholine itself, nicotine can remove acetylcholine from its receptors by affecting the central nervous system (CNS), causing problems such as addiction, the ability to damage genomes, and cancer.[19,40] Also, the presence of more nicotine than acetylcholine along with its receptor causes desensitization of cells to nicotine.[41] For example, nicotine has a greater tendency to α4β2 heteromeric receptors than α7 receptors, which over time

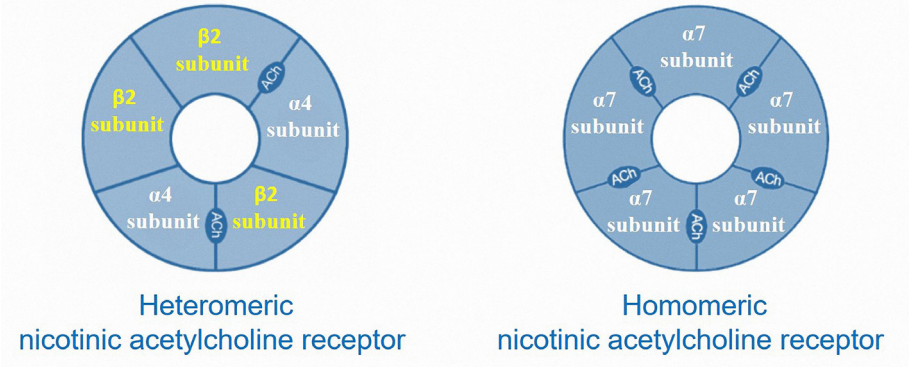

Figure 1. Nicotinic acetylcholine receptors can be homopentamers or heteropentamers. These receptors are the assembly of five subunits forming heteropentameric or homopentameric receptors that are arranged around a central ion pore

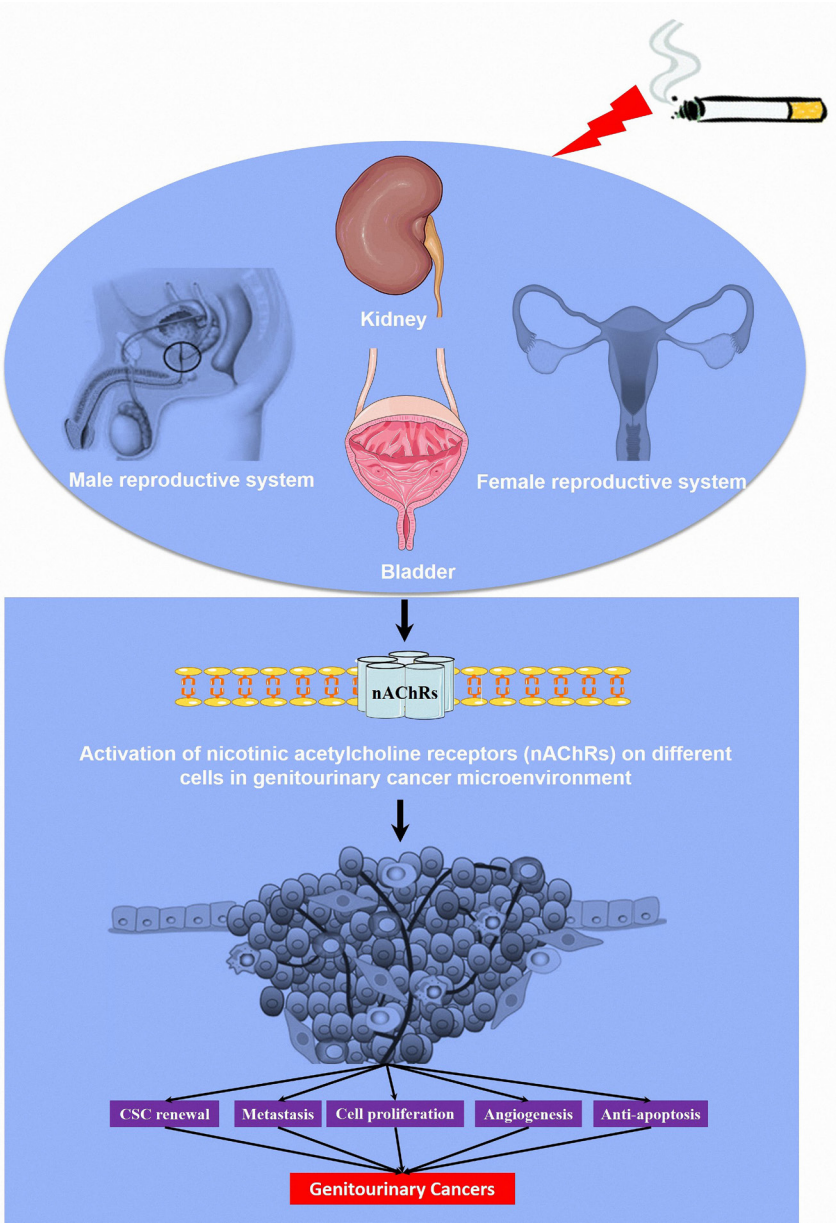

Figure 2. Nicotinic acetylcholine receptors (nAChRs) in genitourinary cancers (GUCs). nAChRs are expressed on different types of the cells in GUC microenvironment, from a wide variety of tissues including kidneys, bladder, as well as male and female reproductive systems. In addition to tumor cells, other cells like cancer stem cells (CSCs), immune cells, and cancer-associated cells such as tumor-associated macrophages and cancer-associated fibroblasts express nAChRs. It has been shown that these receptors promote self-renewal of some subsets of genitourinary CSC and are implicated in tumor metastasis, tumor cell proliferation, angiogenesis, and prevention of apoptosis. Thereby, the activation of nAChRs by smoking-related ingredients can lead to the development and progression of GUCs and the evaluation of these receptors could also be useful in GUCs diagnosis and therapy

and constant exposure to nicotine cause desensitization of α4β2 receptors, while nicotine tendency does not alter the α7 receptor and over time, it can lead to many cancers.[18,42] Cancers that can be caused by nicotine include small-cell and non-small-cell lung carcinomas, head and neck, gastric, pancreatic, gallbladder, liver, colon, breast, cervical, urinary bladder, and kidney cancers.[39,43–47] In other words, nicotine is an addictive but non-carcinogenic substance that performs its biological function through nAChR, b-adrenoreceptors, and/or epidermal growth factor receptor (EGFR) in the motor muscle endplates and the nervous system.[48–50] Consumption of nicotine derivatives in mice causes pulmonary adenocarcinoma (PAC), so it can also cause PAC in smokers.[51] One of the most important and abundant groups of these receptors is α7 and α4β2, in which the α7 receptor is more involved in inflammatory signaling pathways. The distribution of these receptors in the neural and non-neural tissues is very important in their association with various cancers.[52] nAChRs are associated with many cancers, including different types of GUCs, and increasing the expression of each of these receptors can increase the incidence of these cancers. In the next sections, after discussion of the relation between nicotine and its receptors with common GUCs to both genders such as kidney and bladder, we continue with describing the potential effects of nAChR

in male-specific and female-specific cancers.

Kidney cancer

Kidney or renal cancer is a disease in which kidney cells become cancerous and grow out of control. This cancer represents 2–3% of all adult malignancies.[53] Epidemiological studies have demonstrated that cigarette smoking is an independent risk factor in the progression of kidney cancer and the risk of this cancer in smokers is twice more than nonsmokers.[54] Therefore, identifying the novel tumor biomarkers in this smoking-related cancer remains an urgent and essential need. Almost all kidney cancers first appear in the lining membrane of the kidney tubules which is called renal cell carcinoma (RCC). RCC is a heterogeneous disease with the majority of cases categorized into one of two major histological subtypes; 80% are clear cell RCC (ccRCC) and 20% are nonclear cell RCC (nccRCC).[55] RCC is a commonly encountered GUC with over 320,000 patients diagnosed annually and an annual death toll of over 140,000 people in the world. The potential role of nAChR in the genesis of kidney cancer was first hypothesized by Rotola et al. Since then, a few reports with conflicting results have been published on the topic. Gong and colleagues demonstrated the functional expression of α7nAChR in human embryonic kidney cells.[56] Controversy to the stimulatory role of nAChRs in kidney cancer, in another study, it was demonstrated that ACh by targeting α7nAChR on renal macrophages and subsequent activation of cholinergic anti-inflammatory pathway alleviate inflammatory processes, which may play a pivotal role in the management of kidney injury caused by hemorrhagic shock.[57]

Bladder cancer

Bladder cancer (BC) is the ninth most commonly diagnosed cancer in the world, ranks thirteen in overall mortality ranks.[58,59] BC causes significant death in the aging population and is one of the most common GUCs.[60] Unfortunately, there have been poor improvements in the five-year survival rates for BC in over thirty years.[61] Urothelial cell carcinoma (UCC) of the bladder is a type of cancer that typically occurs in the urinary system. It is the most common type of BC and accounts for 95% of diagnosed cases.[62] In the last years, numerous urine-based tests for UCC have been developed and tested in different populations especially in the elderly patients (median diagnosis age of UCC is 73 years). For the urological practice, considering the expression levels of nAChRs would be useful. Cigarette smoke is a major risk factor for BC. Yamamoto et al. demonstrated the up-regulation of nAChRs including α1–α7, β1–β4, ε, and muscarinic acetylcholine receptors (mAChRs) including M1–M5 subtypes mRNA expression in rat bladder by repeated administration of nicotine.[63] In previous studies, it was proved that nicotine by activation of nAChRs expressed in different bladder cells exacerbates the disease of this organ but the exact mechanisms have not been identified. Chen et al. investigate whether there is nicotine-induced bladder epithelial cell proliferation and identify the signaling transduction pathway regulated by nicotine.[64] Their findings revealed that people exposed to nicotine could be at risk for potential deleterious effects, including BC development. The study by Sun and colleagues revealed that nicotine supports stemness and epithelial-mesenchymal transition (EMT) in BC stem cells.[65] These results emphasize that nicotine triggers EMT, leading hence to increased metastasis of BC cells. Therefore, the use of nAChRs as a potential biomarker and blocking their signaling pathways might likely counteract nAChRs-mediated EMT effects on BC pathogenesis.

Prostate cancer

Prostate cancer (PCa) is the second most common cancer in men and the second leading cause of cancer-related death in the USA.[66] Treatment of PCa depends on the anatomic extent of disease, histologic grade, and serum prostate-specific antigen level. Localized PCa is often initially treated with either radical prostatectomy or radiation therapy. Unfortunately, previous studies indicated that PCa recurrence will develop in about half of the patients.[6] Observational studies have suggested a possible connection between nAChR and PCa. However, the association between nAChR and the risk of developing PCa remains to be investigated. The subunit α5 of nAChRs as an important member of the nAChR family is involved in the proliferation and invasion of human PCa cells.[12] Magnon et al. reported that autonomic nerve development contributes to PCa initiation and dissemination.[67] In this study, through the use of animal models of PCa, the authors demonstrated the role of the parasympathetic nervous system (PNS) in PCa development and progression. The results revealed that the cholinergic fibers of the PNS are responsible for the invasion and metastasis of PCa cells by releasing ACh. Considering the potential effects of ACh in activating nAChRs, identifying the exact role of these receptors in PCa may help us in determining the appropriate nAChRs-based therapies to manage the disrupted autonomic mechanisms involved in the progression of prostate tumors.

Testicular and penile cancer

There is evidence of a possible association between nAChR-associated pathways and testicular cancer based on the higher risk of testicular cancer in smoker people.[68] Previous studies designated that, the activation of nAChRs may be involved in tumor growth and metastasis and modulate the formation, metastasis, and recurrence of several types of cancers including testicular cancers.[69] Gu et al. reported that nicotine induces apoptosis in mouse testes.[70] In mouse testes, germ cell apoptosis can be caused by cigarette smoke and lead to declining quality of semen, but the exact molecular mechanisms remain unclear.[70] Similar to testicular cancer, penile cancer is a relatively rare disease representing less than 1% of all malignancies

in Europe and the United States with an estimated incidence ranging from 0.1 to 1.5 per 100.000 men. However, the incidence rate increase in less-developed countries reaches 3.7 per 100.000 men.[71] Epidemiological studies determine that penile cancer was associated with smoking and this behavior was a risk factor with a dose-response relation and remained associated with penile cancer even after adjustment for confounding factors.[72,73] The expression, localization, and function of nAChRs were investigated in the rat corpus cavernosum.[14] The results revealed that the α7 subtype of nAChR is expressed in rat corpus cavernosum and modulates the neurogenic relaxation response to nicotine.[14] Bozkurt et al. indicated that nicotine as a potent activator of nicotinic receptors potentiates the nitrergic relaxation responses of corpus cavernosum tissue via nAChRs.[74] Regarding the evidence about physiologic and pathologic roles of nAChR in penile tissue, it could be concluded that nAChR is expressed corpus cavernosum and it may be involved in the increased risk of penile cancer in smoker patients and active surveillance of their expression could be a novel approach to penile cancer diagnosis and monitoring.

Cervical cancer

Three main types of gynecologic cancer (i.e. any cancer that starts in a woman's reproductive organs) are cervical cancer, ovarian cancer, and uterine cancer. Nowadays, nicotine exposure during pregnancy through cigarette smoking, nicotine replacement therapies, or electronic cigarette use continues to be well-identified gynecologic health problems. Cervical cancer is one of the most common female malignancies in the world with 570,000 cases and 311,000 deaths globally in 2018.[75] Cervical cancer accounts only for 6% of all cancers in the US, but it is the second leading cause of worldwide cancer-related mortality.[75] The majority of cervical cancer deaths occur in developing countries due to poor healthcare facilities. Smoking is one of the major risk factors for cervical cancer. Lane et al. study show that nicotine enhances cellular proliferation of cervical cancer cell lines by up-regulating EGFR, which leads to increased lymphogenic metastasis of cervical cancer. In a most relevant study, Calleja-Macias and colleagues for the explanation of the molecular role of tobacco smoking in cervical carcinogenesis indicated that cholinergic signaling through nAChRs stimulates the proliferation of human CaSki, SiHa, and HeLa cell lines of cervical cancer.[76] Their observations suggest that normal and neoplastic cervical squamous epithelial cells express several combinations of the pentameric nAChRs.[76] This observation indicates that cholinergic signaling under normal physiological conditions and stimulated by nicotine in tobacco users affects epithelial homeostasis and neoplastic progression at the cervix tissue. In addition to nAChRs, Parnell et al. showed that mAChRs in cervical cancer cells affect cell motility via ERK1/2 signaling.[77] Liu et al. indicated that cervical cancer correlates with the differential expression of nAChRs and reveals therapeutic targets. In this study, the results showed that α3, α9, α10, and β4 nAChR subunits were overexpressed in SiHa cells compared with that in normal cells. α9 and α10 nAChR subunits were overexpressed in CaSki cells. Alpha-conotoxins that targeted either α9α10 or α3β4 nAChR were able to significantly inhibit cervical cancer cell proliferation.[78] These findings may provide a basis for new targets for cervical cancer diagnosis as well as targeted therapy.

Ovarian and uterine cancer

Ovarian cancer is the most lethal of all gynecological cancers and is the 5th leading cause of cancer-associated death for women worldwide. It is typically diagnosed after it has progressed to advanced stages, and is among more challenging malignancies to treat.[79] Therefore, identifying novel tumor biomarkers is critical to advancing the diagnosis and treatment of ovarian cancer. Petrik et al. found α2nAChR and α7nAChR expression in ovarian tissues and isolated granulosa cells and suggested that one mechanism by which nicotine may cause the folliculogenesis defects observed in adult female rats was through the induction of apoptosis in granulosa cells and/or oocytes via activation of these receptors.[80,81] Harmych et al. designated that nicotine inhibits MAPK signaling and spheroid invasion in ovarian cancer cells.[16] In this study, the results indicate that nicotine can suppress spheroid invasion and compaction as well as proliferation in ovarian cancer cell lines, and p38 and ERK MAPK signaling pathways are important mediators of these responses.[16] Uterine cancer is a type of cancer that develops from the tissues of the uterus. As the endometrium is the inner lining of the uterus this cancer is also called endometrial cancer. Franks et al. reported that post-menopausal women who smoke exhibit a substantially lowered risk of endometrial cancer.[82] In opposition to most of the studies, some innovative new models provide evidence that smoking may alter fertility through effects on uterine-fallopian tube functions which mediate gamete and conceptus transport. It is of interest that smoking is associated with a decreased incidence of uterine fibroids, endometriosis, and uterine cancer, which may reflect inhibitory effects of smoke constituents on uterine cell proliferation and extracellular matrix interactions.[83]

Discussion

Over the past years, there have been marked advances in our understanding of the molecular interplay within tumor biology. The genitourinary oncology field needs integration of results from basic science and clinical research to improve the current methods for early diagnosis and therapy. Incidences of GUCs have been on the rise and are projected to lead to over 33,000 deaths per year. With the increasing frequency of GU cancers, efforts have been undertaken to increase the efficacy of current therapy options and introduce novel diagnostic and therapeutic targets. However, these efforts require further

optimization and innovation as the burden of these diseases is rising concomitantly.[84] GUCs derive from different types of cells located in the kidney, bladder, prostate, testis, and penis in males and three major gynecologic cancers in females. nAChRs are expressed in both male and female genitourinary tissues and have an important role in genitourinary tissue structure and function. Altogether, α7nAChR, an emerging pharmacological target for a variety of medical conditions, is expressed in most GUC tissues with different effects. This receptor in GUCs represents significant opportunities, especially in the context of recent breakthroughs. A growing body of evidence suggests that in GUCs, for which there is currently no current standard of care or systemic therapy, nAChR-based therapies may slow disease progression. Thus, nAChRs may provide a useful signature for GUCs management and led to changing the current options. The nicotine is used for smoking cessation to relieve withdrawal symptoms and has clinical use in some other conditions.

While the parasympathetic nervous system appears to be involved in the regulation of tumor progression, its exact role is still unclear. The nAChRs are an important regulator of cancer pathways and modulate many cancer-related processes. Recent experimental and clinical investigations indicated its role in proliferation, angiogenesis, metastasis, and apoptosis inhibition. On the other hand, there is mounting evidence showing that α7 subtypes of nAChRs have profound impacts on inflammation control. Activation of these receptors has profound effects on immune responses through the cholinergic anti-inflammatory pathway that mediates primarily by α7nAChR. α7nAChR is an essential regulator of inflammation,[85] and its anti-inflammatory function emerged as a novel therapeutic approach for inflammation-based diseases in recent years. About 20 years passed from the publication of Kevin J. Tracey's report about the role of α7nAChR in controlling inflammation[86] and the introduction of the term "Cholinergic anti-inflammatory pathway" in their influential report.[87] In recent years, the causal relationship between inflammation and different cancer pathogenesis has been a major focus.[88] Recent studies have shown that inflammatory cells infiltrate GUCs and these types of cancers are among tumors that frequently arise as a result of chronic inflammation. It is well documented that, in addition to tumor cells, other tumor-infiltrating immune cells including lymphocytes,[89] monocytes,[90] and macrophages[91] as well as some other microenvironment cells such as fibroblasts[92,93] and endothelial[94] cells express nAChR subunits. These receptors participate in the pathogenesis of many different malignancies. However, the exact mechanisms of nAChR-mediated tumorigenesis are poorly understood. Thus, identifying the association between inflammatory responses and GUCs and the essential role of nAChRs in both of these conditions is helpful to diagnose the diseases.

Conclusion

This review study highlighted the important role of nAChR in GUCs diagnosis and prognosis (Figure 2). It is well-known that the risks among smokers are significantly higher than that of the general population. Based on a review of the literature, although a relationship between nicotine and its receptors and the development of GUCs has been proposed for decades, the results of epidemiologic studies remain inconclusive. Herein, we described the effects of nicotinic receptors and their contribution to GUCs, but the exact underlying mechanisms remain to be elucidated in future studies. In summary, a growing body of evidence suggests that in GUCs, for which there is currently no standard predictive biomarker or systemic care, nAChR-based approaches for diagnosis and treatment may provide efficient therapeutic strategy and slow disease progression.

Conflict of Interest

The authors declare that they have no conflict of interest.

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