Foundation review: α7-Nicotinic receptor antagonists at the beginning of a clinical era for NSCLC and Mesothelioma?

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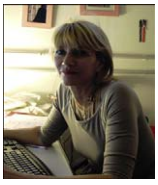
Of the human solid cancers, Non-Small Cell Lung Cancer (NSCLC) and Malignant Pleural Mesothelioma (MPM) display a natural history supporting the concept that they develop from multiple preneoplastic pathways. Recently, new evidence suggested that nicotinic Acetylcholine Receptors (nAChRs) play a significant role in lung cancer predisposition and natural history. This review is based on some translational research aimed at evaluating the potential therapeutic effect of nAChR antagonists on NSCLC and MPM. The background and rationale of this approach are based on the experimental observations that: (a) NSCLC and MPM cells express nAChRs and (b) the activation of these receptors by agonists, namely nicotine, inhibits apoptosis, whereas receptor antagonists have a pro-apoptotic effect.



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Patrizia Russo is in charge of the Lung Cancer Unit at the National Institute for Research on Cancer, Genoa, Italy. In 2006 she became a member of the Italian Consensus Board for the definition of Italian lung cancer guidelines. Since 1987, she has been working in the field of Molecular Pharmacology and in 1987, 1991 and 1997–1998 she attended the Laboratory of Molecular Pharmacology at NCI, NIH (Bethesda, USA). She studied the molecular mechanism of action of caffeine, then the association of antitumor drugs with cytokines. Parts of the latter studies were funded by AIRC (Milan, Italy), CNR (Rome, Italy) and NATO (Bruxelles, Belgium). Since 1995, she has been studying the relationship of sensitivity or resistance to classical and new experimental targeted antineoplastic drugs, receiving a fellowship from FIRC (Milan, Italy) to join the Laboratory of Molecular Pharmacology at NCI, NIH. These studies were supported by EC (Ispra, Italy). Since 2000, she has been studying lung cancer, looking at early detection and the development of new drugs. These studies were supported by the Italian Health Ministry, Fondazione Compagnia di San Paolo (Turin, Italy), Fondazione CARIGE (Genoa, Italy) and Liguria Local Government (Genoa). Starting in 2003, she has been investigating the role of nAChRs in lung cancer biology.

Introduction

Non-Small Cell Lung Cancer (NSCLC) and Malignant Pleural Mesothelioma (MPM) demonstrate great molecular heterogeneity in which several pathways are believed simultaneously and actively to lead to tumorigenesis [1]. Thus, their natural history supported the concept that they develop from multiple preneoplastic pathways. MPM is an aggressive neoplasm of mesothelial cell origin that arises mainly from the pleura and is strongly associated with asbestos exposure [2]. Conventional therapies, such as surgery, radiotherapy and chemotherapy, do not necessarily improve overall survival [3–4]. Lung cancer consists of several histological types in which NSCLC represents 75–85% of the total, it is subclassified into: adenocarcinoma (AD, including the noninvasive type of bronchioloalveolar carcinoma), squamous cell carcinoma (SQ), epidermoid and large cell carcinoma [5]. In AD at least two pathways have been identified: (i) smoking-related and (ii) nonsmoking-related [6]. Recently, genetic variations in a region of chromosome 15 that encompasses a gene implicated in nicotine dependence had been linked to the risk of lung cancer in genome-wide association studies, but data were not definitive as to whether the variants were linked to lung cancer *per se* or to nicotine dependence [7–12]. A recent work [12] reported that the increased risk of lung cancer conferred by the genetic variants might be explained by an increased

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likelihood of nicotine dependence, although some of the results of this study, and a previous study of the variant in subjects who reported that they had never smoked, suggest that the variants may also have a direct role in lung carcinogenesis.

Pharmacology and signaling of nAChRs

nAChRs belong to the ligand-gated ion channel (LGIC) family that includes the excitatory 5HT-3 receptor and the inhibitory receptors for glycine and γ-aminobutyric acid ($GABA_A$ and $GABA_C$) [13]. The nAChR consists of either a homo or heteropentamer composed of the various subunits that have been identified so far (α1–α10; β1–β4) [14,15] and that are arranged symmetrically around an axis perpendicular to the membrane, thus delineating the ionic pore. The composition and stoichiometry of the subunits constituting the pentamer may have a profound impact on receptor pharmacology, cation selectivity, desensitization kinetics and spatial distribution. All nAChR subunits share a homologous structure, with a large extracellular domain, four transmembrane regions (M1–M4) structured in α-helices, a large cytoplasmic domain between M3 and M4 and, finally, a short extracellular C-terminal tail [16].

The cognate ligand for the nAChRs is nicotine, an agonist interacting with various affinities, from 1 to 130 μM, depending upon the different neuronal receptor subtypes [17,18]. The presence of β2 and β4 subunits in the receptor pentamer seems to be correlated with high and low affinity for nicotine, respectively.

On the contrary, as an allosteric receptor (see the below paragraph), nAChR may undergo rapid conformational transitions from a resting basal state to an active or desensitized state. Application of nicotine initially provokes the stabilization of the receptor in a high affinity, open state followed by a progressive stabilization of a closed, desensitized state [19]. In the case of smoking behavior, long exposure to a low concentration of nicotine favors receptor desensitization.

Mutational and photo-affinity labeling experiments identified the agonist binding site at the interface of the extracellular regions of the principal α and the complementary non-α subunits, whereas the transmembrane segment M2 is the major contributor to the pore domain [13]. The structural coupling between the extracellular and the pore domains provides efficient transduction between agonist binding and the ion channel gating. Recently, the resolution of the crystallographic structures of a protein homologous to the extracellular domain of nAChR, the acetylcholine binding protein (AChBP) either alone or complexed with various ligands, in addition to numerous biochemical studies on the ligand binding sites, allowed better understanding of how various ligands interact with different nAChRs and revealed, at the molecular level, the fundamental events underlying the receptor activation [20–24].

The nicotine-binding site was initially studied using structural models of the extracellular domain of nicotinic receptors and then the crystallographic structure of the AChBP–nicotine complex [25]. Even if the nicotine-binding pocket is similar to those determined for acetylcholine or epibatidine, involving mainly aromatic and hydrophobic contacts, the specific binding of nicotine is due to additional hydrogen bonds with the receptor and a closer packing of the aromatic groups [25]. These subtleties in nicotine interaction compared to other agonists were confirmed by a physical chemistry approach using unnatural amino acid mutagenesis combined with computational modeling studies [26].

Nicotine activates different subtypes of nAChR, inducing a complex pattern of mixed sympathetic and parasympathetic responses. The stimulation, desensitization and upregulation of these receptors by nicotine seem to be responsible for diverse physiological effects targeting the cardiovascular [27,28], pulmonary (as we will see in the next chapters), endocrine [29] and central nervous systems [16]. Of course, one of the most studied effects of nicotine is its smoking-related addictive property [30–32].

Allosteric modulation

Introduced by Wyman and colleagues in 1965 [33], the allosteric concept refers to the assumption that proteins could exist in multiple conformational states and that binding of allosteric ligands alters the energy barriers or 'isomerization coefficients' between various states, preferentially stabilizing the protein in a given conformation. The site occupied by the natural ligand, which is typically at the interface between subunit protomers, is called 'the orthosteric site'. Allosteric sites are distinct from the orthosteric site and can be localized elsewhere on the protein. Binding of the ligand at the orthosteric site stabilizes the protein in the active state, whereas binding of an effector at an allosteric site alters the overall properties by modifying the energy barriers, represented by isomerization coefficients, between one or more states. In the specific case of the nAChR, agonists are ligands that preferentially stabilize the receptor in the active open state, whereas competitive antagonists are ligands that stabilize the protein in a closed conformational state. Thus, endogenous ligands, such as acetylcholine, bind at the orthosteric site, whereas all the molecules that bind elsewhere on the nAChR subunit(s) act via allosteric interactions. Bertrand and Gopalakrishnan, in a recent review, outlined extensively the principles of the allosteric concept and summarized the profiles of novel compounds that are emerging as allosteric modulators at the α7- and α4β2-nAChR subtypes [34].

Ca^{2+} permeability of nAChR

The nAChR channels are permeable to cations, including Ca^{2+}. Ca^{2+} entry through nAChR channels modulates several Ca^{2+}-dependent cellular processes, such as neurotransmitter release, synaptic plasticity and cell motility. Two different classes of neuronal nAChR may be identified according to their Ca^{2+} permeability, which correlates with other pharmacological and structural properties: (i) neuronal nAChRs containing subunits (α7–α9) able to bind α-bungarotoxin (α-BTX) and form homopentameric channels (α-BTX nAChR), exhibiting the highest measured Ca^{2+} permeability values [35]; (ii) heteropentameric, non-α-BTX-sensitive nAChRs (non-α-BTX nAChR), always comprising at least one α (out of α2–α6) and one β (out of β2–β4) subunits, with lower measured Ca^{2+} permeability [36]. Studies indicate a functional correlation between the activation of α7-nAChR and Ca^{2+}-dependent cellular processes, such as neurotransmitter release, synaptic plasticity, cell growth, migration and survival.

A recent study by Gilbert *et al.* [37] showed that the Ca^{2+} transients were predominantly due to the opening of plasma membrane α7-nAChR, because the signals were (a) evoked by nicotine, (b) sensitive to two α7-specific nAChR inhibitors