Abstract. Background: Cholinergic receptors are expressed in small cell lung cancer (SCLC); however, the distinct functions of muscarinic cholinergic receptor 3 (mAChR3) and the nicotinic cholinergic receptor (nAChR) in SCLC have not yet been completely elucidated. Materials and Methods: RT-PCR and Western blotting were used to investigate the expression of cholinergic receptors. Flow cytometry was used to detect the integrin expression. Cell proliferation, adhesion and migration assays were carried out in vitro to determine the roles of the cholinergic receptors in SBC3 human SCLC cells. Results: Both mAChR3 and nAChR were expressed in the SBC3 cells. Acetylcholine iodide (Ach) stimulated SBC3 cell proliferation, adhesion and migration toward fibronectin (Fn). The mAChR3 antagonist, 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP), or the nAChR antagonist, mecamylamine hydrochloride (Meca), inhibited SBC3 cell proliferation in the presence or the absence of exogenous Ach. 4-DAMP abrogated cell adhesion and migration toward Fn induced by Ach, while Meca had no effect. Interestingly, Ach did not alter Fn receptor (αvβ1 or α5β1 integrin) expression, while anti-β1 integrin antibody or anti-αv and anti-α5 integrin antibody completely abrogated cell adhesion to Fn induced by Ach. Conclusion: Both mAChR3 and nAChR are expressed in SCLC. SBC3 cell proliferation is regulated in vitro through both cholinergic receptors. In contrast, SBC3 cell migration and adhesion toward Fn are modulated only by mAChR. Moreover, the stimulatory effects of Ach on cell adhesion and migration through mAChR3 are presumably modulated by functional alteration of αvβ1 and α5β1 integrin, but not by any variation in their expression. The mAChR3 antagonist may therefore be a beneficial therapeutic modality for SCLC patients, especially those with chronic obstructive pulmonary disease (COPD) as a comorbidity.

Lung cancer is one of the most common fatal malignancies in the world. Annual global lung cancer deaths exceeded 1,000,000 in the year 2000 and are expected to exceed 2,000,000 by the year 2020 or 2030 (1). Meanwhile, 20-30% of patients diagnosed with lung cancer have spirometric chronic obstructive pulmonary disease (COPD) (2). Small cell lung cancer (SCLC), which accounts for 15% to 25% of all lung carcinomas, is distinguished from non-small cell lung cancer (NSCLC) by its rapid doubling time and early development of widespread metastases. Surgery has a very limited role in SCLC treatment, especially for those patients who have COPD at the same time. More effective drugs to treat this serious malignancy are essential.

The cholinergic system plays a critical role not only in mammalian neuronal tissues but also in non-neuronal tissues. Muscarinic cholinergic receptor 3 (mAChR3) has been detected in the airway smooth muscle (ASM) and bronchial epithelial cells, fibroblasts as well as myofibroblasts and plays various pathophysiological roles in the airways (3-5). The mAChR3 antagonist has been used clinically to treat COPD for a long time. The role of cholinergic signaling in neoplasia has recently received relatively more attention.

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Key Words: Muscarinic cholinergic receptor 3 (mAChR3), nicotinic cholinergic receptor (nAChR), small cell lung cancer (SCLC), β1 integrin.
Most studies have been concentrated on nAChR due to the close relationship between tobacco and lung cancer. Nicotine stimulates lung cancer carcinogenesis, proliferation and angiogenesis and also inhibits lung cancer apoptosis induced by chemotherapeutic drugs through the nAChR (6-9). Even though mAChR3 is expressed in some SCLC cell lines (10), the function of mAChR3 in SCLC is still not clearly understood. Furthermore, the distinct roles of mAChR3 and the nAChR in SCLC have not been completely elucidated. Therefore, the aims of this study were to determine whether mAChR3 and the nAChR were expressed in SCLC and to investigate the functions of these two receptors.

Materials and Methods

Cell culture. The SBC3, human SCLC cell line, was kindly provided by Dr. I. Kimura (Okayama University, Okayama, Japan) and the H82, human SCLC cell line, was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in PRMI1640 medium (Kohjin Bio, Tokyo, Japan) containing 10% (v/v) fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37˚C in a 5% CO2 atmosphere.

Reagents. The primary antibodies included anti-mAChR3 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-β-actin monoclonal antibody (Abcam, Cambridge, UK) and anti-αv, α2, α5, β1 and β3 integrin antibodies (Chemicon International Inc., Temecula, CA, USA). The secondary antibodies included anti-rabbit horseradish peroxidase (HRP) antibody, anti-mouse HRP antibody (Amersham Bioscience, Bucks, UK) and anti-mouse IgG fluorescein isothiocyannate (FITC) antibody (Chemicon International Inc.). The non-specific cholinergic receptors agonist acetylcholine iodide (Ach) was purchased from Wako, Osaka, Japan. The mAChR3 antagonist 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP), nAChR antagonist mecamylamine hydrochloride (Meca) and MTT assay reagent were purchased from Sigma (St Louis, MO, USA). Insulin-transferrin-selenium (ITS) was purchased from Mediatech Inc., Manassas, VA, USA.

Reverse transcriptase-polymerase chain reaction (RT-PCR) for cholinergic receptor mRNAs. RT-PCR was used to investigate the expression of mAChR3, mAChR subunits α3, α5, α7, β2 and β4 mRNAs in the SBC3 and H82 cells. The RNAs were isolated from the SBC3 and H82 cells with TRIzol Reagent (Invitrogen, San Diego, CA, USA). The mAChR gene has no introns, and contamination of the RNA preparation by genomic DNA would cause false-positive results. Therefore, the RNA preparations were treated with DNase to remove contaminating genomic DNA before the RT reaction using Deoxyribonuclease I, Amplification Grade (Invitrogen) according to the manufacturer’s instructions. cDNAs were synthesized by reverse transcriptase. β-Actin with reverse transcriptase was used as the positive control. β-Actin without reverse transcriptase was used as negative control for amplified residual genomic DNA. The primers for PCR are shown in Table I (10). PCR amplification was performed using a Gene Amp RNA PCR kit (Applied Biosystems, Branchburg, NJ, USA) according to the manufacturer’s instructions.

Western blotting for mAChR3 protein. Western blotting was used to investigate the expression of mAChR3 proteins in the SBC3 and H82 cells. Subconfluent SBC3 and H82 cells were homogenized with ice cold lysis buffer containing 50 mM Tris-HCl (pH 7.2), 1% TritonX-100, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 0.1 mM Na3VO3 and 10 μg/ml protease inhibitor cocktail (Thermo Fisher Scientific, Rockford, IL, USA). The homogenates were sonicated for 30 sec and centrifuged at 12,000 g for 20 min at 4˚C. The supernatants were separated on 10% acrylamide gels and transferred to nitrocellulose membranes with electroblotting at 4˚C overnight. The membranes were blocked for 1 h with PBS-T (0.1% Tween-20) containing 5% dry milk and subsequently incubated with rabbit anti-mAChR3 polyclonal antibody (1:800) or mouse anti-β-actin monoclonal antibody (1:3000) for 1 h at room temperature. After 3 rinses with PBS-T, the membranes were incubated with anti-rabbit HRP secondary antibody (1:5000) or anti-mouse HRP secondary antibody (1:4000) for 1 h. The mAChR3 proteins were determined with enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham Biosciences). The intensity of the detected bands was analyzed with the Multi-Gauge Ver3.0 software (Fuji Photo Film Co., Ltd, Kanagawa, Japan).

Table I. RT-PCR primers.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Length (bp)</th>
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<tbody>
<tr>
<td>mAChR3</td>
<td>S: 5'-TGGAACAACAAATGATGCTGC-3'</td>
<td>432</td>
</tr>
<tr>
<td></td>
<td>A5: 5'-CTTITCTCCGTTAATGTGATCGT-3'</td>
<td></td>
</tr>
<tr>
<td>α3 nAChR</td>
<td>S: 5'-GTTGTITCTGCTGATCAGCTGACCAC-3'</td>
<td>756</td>
</tr>
<tr>
<td>α5 nAChR</td>
<td>S: 5'-ATTGGGAGATTTGTGATGATGCA-3'</td>
<td>685</td>
</tr>
<tr>
<td>α7 nAChR</td>
<td>S: 5'-CCCTGCGCAATGTGACGTC-3'</td>
<td>414</td>
</tr>
<tr>
<td>β2 nAChR</td>
<td>S: 5'-TGTTGCTCATCACCTCGTACTG-3'</td>
<td>307</td>
</tr>
<tr>
<td>β4 nAChR</td>
<td>S: 5'-GGCCATCTCGTCTCTTACC-3'</td>
<td>677</td>
</tr>
<tr>
<td>β-actin</td>
<td>S: 5'-AGAAAAATCTGGACCACCAAC-3'</td>
<td>550</td>
</tr>
<tr>
<td></td>
<td>A5: 5'-AGAAAGGAGGCTGGAAGAG-3</td>
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</table>

Most studies have been concentrated on nAChR due to the close relationship between tobacco and lung cancer. Nicotine stimulates lung cancer carcinogenesis, proliferation and angiogenesis and also inhibits lung cancer apoptosis induced by chemotherapeutic drugs through the nAChR (6-9). Even though mAChR3 is expressed in some SCLC cell lines (10), the function of mAChR3 in SCLC is still not clearly understood. Furthermore, the distinct roles of mAChR3 and the nAChR in SCLC have not been completely elucidated. Therefore, the aims of this study were to determine whether mAChR3 and the nAChR were expressed in SCLC and to investigate the functions of these two receptors.
In vitro cell proliferation assay. SBC3 cells were used for the
remaining experiments. Concentrations of 10–6, 10–5, 10–4 and 10–3
M Ach, 10–8, 10–7, 10–6 and 10–5 M 4-DAMP and 10–7, 10–6, 10–5
and 10–4 M Meca were used. The SBC3 cells were seeded at 2000
cells/well in treated 96-well plates (Corning Inc., Corning, NY,
USA) maintained with RPMI1640 medium containing 10% (v/v)
FCS for 24 h. The medium was removed and replaced with 100 μl
RPMI1640 containing 1% (v/v) ITS. The cells were incubated in
serum free medium for 24 h to synchronize the cell cycle. Then
another 100 μl serum-free medium containing the agonist/antagonist
under investigation was added. The antagonist was added 30 min
before Ach. At this time, the cells in a duplicate plate were given
100 μl serum-free medium and a MTT assay was used to determine
live cell numbers before treatment with the agonist/antagonist as
measured by the absorbance (OD) at 550 nm/650 nm. After 24, 48
or 72 h culture, the remaining cells were assayed to yield post-
agonist/antagonist cell numbers using the same method. All the
experiments were performed in triplicate.

In vitro cell migration assay. SBC3 cell migration was detected
using the Boyden blind well chamber (Neuroprobe Inc.,
Gaithersburg, MD, USA) as previously described (11). Twenty
seven μl of human Fibronectin (Fn) (Roche, Indianapolis, IN, USA)
(20 μg/ml), used as a chemoattractant, was placed into each bottom
well. The assay used 8 μm pore polycarbonate membranes
(Neuroprobe Inc.). The cells at a density of 1x10⁶/ml were
pretreated with 4-DAMP or Meca 30 min before Ach. A 50 μl cell
suspension treated with various concentrations of Ach (10–7, 10–6,
10–5, 10–4 M), 4-DAMP (10–7, 10–6, 10–5 M) or Meca (10–6, 10–5,
10–4 M) were added to each top well. The cells were allowed to
migrate for 6 h. Any cells that had not migrated were scraped off
the upper surface of the membrane and then the membranes were
air-dried. The cells were stained using a Diff-Quick staining kit
(Kokusai-Shiyaku, Kobe, Japan). Wells containing serum free
medium were used as negative controls. Migration was assessed by
counting the number of cells in five independent high-power fields
(HPF) (400×) including one centric and four ambient fields with
light microscope and calculating the mean number. All the
experiments were performed in triplicate.

Flow cytometric analysis for integrin expression. The following
procedures were performed as previously described (12). Briefly the
adherent SBC3 cells were detached with 0.05% EDTA in PBS and
incubated with anti-human αv, α2, α5, β1 and β3 integrin antibodies
in PBS at 4˚C for 1 h. After washing, the cells were incubated with anti-mouse IgG FITC antibody at 4˚C for 30 min.
Propidium iodide (PI; Sigma) was added at a final concentration of
10 μg/ml. A flow cytometric analysis was performed with a
FACScan™ (Becton-Dickinson Co., Mountain View, CA, USA).
Detached cells were treated with 10–5 M 4-DAMP or 10–4 M Meca
30 min before 10–4 M Ach and cultured for 6 h. The same method
was used to detect changes in the expression of αv, α5 and β1
integrin.

In vitro cell adhesion assay. The following procedure was
performed as described previously (13). Briefly, non-treated 96-well
plates were coated with Fn (20 μg/ml) or bovine serum albumin
(BSA) (10 mg/ml) in PBS overnight at 4˚C. The detached SBC3
cells were treated with 10–5 M 4-DAMP, 10–4 M Meca and 5 μg/ml
anti-human αv integrin antibody, α5 integrin antibody, β1 integrin
antibody or αv and α5 integrin antibody for 30 min before 10–4 M
Ach was added. The cells were then seeded at 5x10⁵ cells/well in
the coated plates and cultured for 2 h. After washing, the adherent
cells were counted in five high-power fields (×400) and the mean
number of cells was calculated. All the experiments were performed
in triplicate.

Statistical analysis. The Statistical Product and Service Solution 12.0
software package (SPSS Inc., Chicago, IL, USA) was used for the
analysis. The data were calculated as the mean±S.D. Comparisons
between two groups were conducted using the Student’s t-test.
P<0.05 was considered to be statistically significant.

Results
Expression of mACHR3 and nACHR mRNAs by RT-PCR
analysis. As shown in Figure 1A, the specific band for
mACHR3 was detected at the predicted size of 430 bp (lane
9) in the SBC3 cells. nACHR α5 subunit expression (lane 5)
was observed at the predicted size of 685 bp. The H82 cells
showed a specific band for mAChR3 at 430 bp (Figure 1B,
lane 4), but the expression of mAChR3 was much weaker
than that in the SBC3 cells. Specific bands for the nACHR
α3 (lane 8), α5 (lane 6), α7 (lane5) and β4 (lane 9) subunits
were detected at the predicted sizes of 756 bp, 685 bp, 414
bp and 677 bp.
Expression of mAChR3 protein by Western blotting analysis. As shown in Figure 2A, the bands corresponding to mAChR3 were detected at 55 kDa. Both SBC3 and H82 expressed mAChR3. The relative expression of mAChR3 normalized with β-actin in the SBC3 cells was 2.65-fold higher than that in the H82 cells (Figure 2B).

mAChR3 and nAChR modulation of proliferation in SBC3 cells. According to the RT-PCR and Western blotting results, mAChR3 mRNA and protein were predominantly expressed in the SBC3 cells, but not in the H82 cells. Moreover, few nAChR subunits except α5 seemed to be expressed in the SBC3 cells. Therefore, the SBC3 cells were used in the experiments to investigate mainly the function of mAChR3 in vitro. As shown in Figure 3A, Ach stimulated SBC3 cell proliferation in a dose-dependent manner. Treatment with 10^{-4} M and 10^{-3} M Ach significantly stimulated SBC3 cell growth at 48 h and 72 h (p<0.01). As shown in Figure 3B, SBC3 cell proliferation induced by Ach was inhibited by 4-DAMP in a dose-dependent manner. Treatment of the cells with 10^{-5} M 4-DAMP inhibited SBC3 cell proliferation induced by Ach at 48 h (p<0.05). Treatment with 10^{-6}, 10^{-7} (p<0.05) and 10^{-5} M (p<0.01) 4-DAMP inhibited cell proliferation induced by Ach at 72 h. To observe whether 4-DAMP alone could inhibit SBC3 cell proliferation, cells were exposed to various concentrations of 4-DAMP without
Figure 4. Role of mAChR3 and nAChR in migration in SBC3 cells. (A) Effect of Ach on cell migration. 20 μg/ml of fibronectin (Fn) was used as chemoattractant. *p<0.01 vs. Fn. (B) The effect of 4-DAMP on Ach stimulated migration. *p<0.05, **p<0.01 vs. 10–4 M Ach. (C) Effect of Meca on cell migration induced by Ach. *p<0.01 vs. 10–4 M Ach. (D) One representative chemotaxis assay of SBC3 cell migration. D1, Fn; D2, 10–4 M Ach; D3, 10–5 M 4-DAMP and 10–4 M Ach; D4, 10–4 M Meca and 10–4 M Ach (×400).
exogenous Ach. As shown in Figure 3C, 4-DAMP alone dose-dependently inhibited the cell proliferation in comparison to the control. Treatment with $10^{-5}$ M and $10^{-6}$ M 4-DAMP inhibited cell proliferation at 48 h ($p<0.01$) and the inhibitory effect of $10^{-5}$ M 4-DAMP was stronger than $10^{-6}$ M 4-DAMP at 72 h ($p<0.01$ vs. $p<0.05$). The role of nAChR in cell proliferation was also investigated. Figure 3D shows that $10^{-4}$ M Meca inhibited SBC3 cell proliferation induced by Ach at 72 h ($p<0.05$). Treatment with $10^{-4}$ M Meca alone inhibited SBC3 proliferation at 48 h ($p<0.05$) and 72 h ($p<0.01$) (Figure 3E).

**Effect of mAChR3 and nAChR on migration in SBC3 cells.**

No cells migrated on the membrane without Fn in the lower chamber (negative control). The cells showed a significant migration with 20 μg/ml Fn in the lower chamber (Fn). Ach increased cell migration towards Fn in a dose-dependent manner and $10^{-4}$ M Ach increased cell migration by ~3-fold in comparison to Fn alone (Figure 4A). Cell migration stimulated by Ach was almost completely blocked by pretreatment with $10^{-5}$ M or $10^{-6}$ M 4-DAMP ($p<0.01$; Figure 4B). However, none of the tested concentrations of Meca showed an inhibitory effect on the SBC3 cell migration.
induced by Ach (Figure 4C). Photographs of one representative migration assay are shown in Figure 4D.

Expression of integrin in SBC3 cells. Integrin expression in the SBC3 cells was detected using flow cytometry. The αv and β1 integrin were predominantly expressed in the SBC3 cells and α5 was minor (Figure 5).

Effect of mAChR3 and nAChR on adhesion to Fn in SBC3 cells. SBC3 cell adhesion to Fn was investigated using Fn coated 96-well plates to confirm the effect of the cholinergic receptors on cell adhesion. As shown in Figure 6A, 10^{-5} M Ach significantly increased SBC3 cell adhesion to Fn (p<0.01). This effect was totally abrogated by 10^{-5} M 4-DAMP (p<0.01), however 10^{-4} M Meca had no effect. The cell adhesion to Fn enhanced by Ach was partially abrogated by 5 μg/ml anti-αv or anti-α5 integrin antibody (p<0.05) and totally abrogated by 5 μg/ml anti-β1 integrin antibody (p<0.01) or 5 μg/ml anti-αv and anti-α5 integrin antibody (p<0.01; Figure 6B). The SBC3 cells showed no binding to BSA (data not shown).

Effect of Ach in the presence or the absence of 4-DAMP or Meca on αv, α5 and β1 expression in SBC cells. The cells treated with Ach in the presence or the absence of 4-DAMP or Meca were subjected to flow cytometric analysis for αv, α5 and β1 expression to investigate whether the cholinergic system influences integrin expression. As shown in Figure 7, αv, α5 and β1 expression was not altered after the cells were treated with Ach, 4-DAMP or Meca.

Discussion

As confirmed with RT-PCR and Western blotting both SBC3 and H82 SCLC cell lines expressed mAChR3 and nAChR. However, only the α5 nAChR subunit was found in the SBC3 cells. The overall structure of nAChRs is a homo- (α7 or α9) or a hetero-pentamer composed of the various subunits (α2-α10, β2-β4) that have been identified (14). Alone, the α5 subunit cannot constitute the functional nAChR. However, we still believe that the nAChR containing the α5 subunit in the SBC3 cells is a functional receptor, because the nAChR antagonist, Meca, inhibited SBC3 cell proliferation. This result suggested that other nAChR subunits should be expressed in the SBC3 cells to form a functional hetero-pentamer.

Ach, a non-specific agonist of cholinergic receptors, stimulated cell proliferation through mAChR and nAChR, because both 4-DAMP, the mAChR3 antagonist, and Meca, the nAChR antagonist, inhibited the in vitro cell proliferation induced by Ach in the SBC3 cells. Since there are reports indicating that Ach can be synthesized by non-neuronal tumor cell lines (15, 16), the effect of 4-DAMP or Meca
alone on cell proliferation was investigated, revealing that 4-
DAMP or Meca also inhibited SBC3 cell proliferation in the
absence of exogenous Ach. These results indicated that Ach
secretion from the SBC3 cells exerted an autocrine effect on
SBC3 cell proliferation and both mAChR3 and nAChR were
functional receptors involved in cell growth in the SBC3
cells. These findings are still controversial. In fact, Williams
demonstrated that activation of mAChR3 inhibited
proliferation in SCLC (17). In contrast, Song et al.
reported the opposite results in SCLC and found that activation of
mAChR3 also could stimulate squamous cell lung cancer
proliferation (18, 19). Recently several studies indicated that
mAChR3 was expressed in some other cell lines, e.g.,
prostate cancer cells, murine mammary adenocarcinoma and
human colon cancer and activation of mAChR3 in these cell
lines could stimulate cell proliferation (20-22).

Previous studies of the effects of cholinergic systems on
cell migration are inconsistent. The cholinergic receptor
agonist, carbachol, did not influence cell migration in
HaCaT, a human keratinocyte cell line (23). Carbachol
increased the chemotactic activity of SK-Mel 28 human
melanoma cells and that effect could be blocked by atropine
(24). However, carbachol inhibited cell migration in Chinese
hamster ovary (CHO) cells (25). No previous studies have
addressed the effect of mAChR3 on lung cancer cell
migration. SBC3 cell migration was increased significantly
with Fn as a chemoattractant. Ach enhanced cell migration
toward Fn in a dose-dependent manner and this effect was
blocked by pretreatment cells with 4-DAMP; however, Meca
had no effect on cell migration induced by Ach, thus only
mAChR3 modulated cell migration in the SBC3 cells. Fn is
an extracellular matrix glycoprotein that binds to membrane-
spanning receptor proteins called integrins (26). Thus mAChR3 may modulate SBC3 cell migration toward Fn through integrin receptors. In the SBC3 cells, αv and β1 integrin were predominantly expressed and α5 was slightly expressed suggesting that αvβ1 could be the principal receptor for Fn in SBC3 cells, although α5β1 is also expressed. While α5β1 integrin is a classical receptor for Fn, αvβ1 integrin was first discovered in the neuroblastoma cell line IMR32 (27). Subsequently, αvβ1 was found in SCLC cell lines and squamous carcinoma cells (28, 29). One function of the receptor is to modulate adhesion and migration to Fn. The mAChR3 antagonist, 4-DAMP, completely inhibited the SBC3 cell adhesion to Fn induced by Ach, while the nAChR antagonist, Meca, did not (Figure 6A). Additionally the anti-β1 or anti-αv and α5 integrin antibodies completely abrogated SBC3 adhesion to Fn induced by exogenous Ach, while the anti-αv or anti-α5 antibody partially blocked adhesion (Figure 6B), indicating that the modulatory role of mAChR3 in SBC3 cell binding to Fn is mediated by β1 containing integrins (αvβ1 and α5β1). These findings suggested the hypothesis that αv, α5 and β1 integrin expression would be altered by treatment with Ach, 4-DAMP and Meca. However, none of them was altered after stimulation or inhibition of the cholinergic receptors in the SBC3 cells. These results indicated that the ability of mAChR3 to regulate cell adhesion and migration to Fn was mediated by functional alteration of the β1 containing integrins (αvβ1 and α5β1), but not their expression variation. In fact, a study by Quigley et al. found that anti-β1 integrin antibody inhibited carbachol induced adhesion of the SCLC cell line, SCC-9 to collagen type IV, while carbachol did not alter expression of β1 integrin (30), thus functional alteration of β1 integrin influenced SCLC cell adhesion, which was consistent with the current result. However, few studies have investigated nAChR and SCLC cell migration, although nicotine, by binding to nAChR was found to stimulate the migration of the lung adenocarcinoma cell line, H1299 (31). This report contradicted the current finding that the nAChR antagonist, Meca, did not inhibit SBC3 cell adhesion and migration induced by Ach. This discrepancy could be explained by a different nAChR expression pattern.

In summary, both mAChR3 and nAChR were expressed in the SCLC cell line SBC3. Blockage of mAChR3 inhibited SBC3 cell proliferation, adhesion and migration. Blockage of nAChR inhibited cell proliferation, but did not affect cell adhesion and migration. The ability of mAChR3 to regulate cell adhesion and migration appears to be mediated by functional alteration of αvβ1 and α5β1 integrin, but not by a variation in their expression.

The side-effects of nAChR antagonists in the neuromuscular junction limit its clinical therapeutic application in lung cancer. The current finding that mAChR3 modulated SCLC cell proliferation, adhesion and migration is encouraging. Inhibition of proliferation, adhesion and migration following mAChR3 blockage may suppress both the progression of primary tumors and formation of newly metastatic tumors. The mAChR3 antagonist could not only alleviate bronchoconstriction and mucus secretion, but also inhibit tumor progression. This therefore provides a beneficial therapeutic option for SCLC patients, especially those with COPD.

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References


