Acetylcholine Is Synthesized by and Acts as an Autocrine Growth Factor for Small Cell Lung Carcinoma

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ABSTRACT

It is well established that small cell lung carcinomas (SCLCs) express receptors for acetylcholine (ACh) and that stimulation of these receptors by nicotine or other cholinergic agonists stimulates cell growth via activation of nicotinic cholinergic receptors (nAChRs) and/or muscarinic cholinergic receptors (mAChRs). The aim of this study was to determine whether SCLC cells synthesize and secrete ACh and respond to endogenous ACh to create a functioning cholinergic autocrine loop. Reverse transcription-PCR was used to screen a panel of SCLC cell lines for components of cholinergic signaling. Choline acetyltransferase (ChAT) and the vesicular ACh transporter (VACHT), as well as α3, α5, α7, β2, and β4, nAChR subunits and M3 and M5 mAChRs, were found to be present in most of the SCLC cell lines tested. Real-time PCR showed that mRNA levels for ChAT, VACHT, and α7 and β2 nAChR subunits varied significantly among different SCLC cell lines tested. The H82 cell line was found to express the highest levels of ChAT, and that cell line was chosen for additional studies of ACh release and cell proliferation. ACh was easily detectable in H82 cell culture media, and levels of ACh were increased by the acetylcholinesterase inhibitor neostigmine. Vesamicol, an inhibitor of VACHT, and hemicholinium-3, an inhibitor of choline transport, both reduced H82 cell ACh basal release in a dose-dependent manner. In parallel with the reductions of ACh release, vesamicol and hemicholinium-3 also decreased H82 cell proliferation. H82 cell proliferation was also inhibited by the muscarinic and nicotinic antagonists atropine and mecamylamine, respectively, in dose- and time-dependent manners. Finally, archival cases of SCLC were screened by immunohistochemistry for expression of ChAT. Thirteen of 26 tumors screened were positive for ChAT. These findings demonstrate that SCLC can synthesize, secrete, and degrade ACh and that released ACh stimulates SCLC cell growth. Identification of this new autocrine loop provides a potential new target for therapeutic intervention.

INTRODUCTION

Global lung cancer deaths were over 1,000,000 for the year 2000 and are projected to increase to 2,000,000 per annum by 2020–2030 (1). SCLC3 accounts for 20–25% of all new cases of primary lung cancer. In addition to morphological characteristics, SCLC is distinguished from non-SCLC by its rapid tumor doubling time, high growth fraction, and early development of widespread metastasis (2). Overall, survival beyond 5 years occurs in only 3–8% of all patients with SCLC because relapse occurs within 2 years despite initial responses to chemotherapy and radiotherapy (3). Thus, there is clear need for further understanding of the biology of SCLC and development of new therapeutic approaches for SCLC treatment.

The neuroendocrine nature of SCLC is well established, as is the concept of growth regulation of SCLC by autocrine growth factors such as gastrin-releasing peptide (4, 5). SCLC and some non-SCLCs secrete a variety of neuropeptides, and many of these act as growth factors (6, 7). The concept of autocrine growth factors has been extended to the secretion of ligands for tyrosine- and threonine-kinase-linked receptors such as basic fibroblast growth factor (bFGF, FGF-2; Refs. 8, 9), epidermal growth factor (EGF; Ref. 9) and transforming growth factor β-1 (10). Therapeutic approaches derived from this have included monoclonal antibodies against the epidermal growth factor receptors (11), broad spectrum neuropeptide antagonists (12), and inhibitors of tyrosine kinases and phosphatases (13). Thus, autocrine growth factors can regulate SCLC growth and are potential therapeutic targets.

Multiple reports have established that SCLC cells express mAChRs and mAChRs (14–17) and that the activation of mAChR and/or mAChR with nicotine (18–20), ACh (19), and muscarine (19, 20) stimulates the growth of SCLC cells. Recent reports that a variety of cell types in normal lung synthesize ACh (21–23) have led us to hypothesize that lung cancers, similarly, may make ACh and that, therefore, SCLC growth may be regulated by a cholinergic autocrine loop.

In cholinergic neurons, the neurotransmitter ACh is synthesized from choline and acetyl-CoA by ChAT (24) and is then translocated into synaptic vesicles by the VACHT (25). In neurons, choline for synthesis of ACh is transported by a specific high-affinity choline transporter: CHT1 (26, 27). If SCLCs synthesize ACh, then ChAT must be present and the other components of neuronal cholinergic signaling may be present. If a cholinergic autocrine loop is present in SCLC, then interruption of ACh synthesis and signaling should modify growth of SCLC. In this article, we show that SCLC tumors and cell lines express ChAT and that SCLC cell lines synthesize VACHT, CHT1, mAChR, and mAChR and synthesize ACh and that interruption of cholinergic signaling affects cell growth.

MATERIALS AND METHODS

Cell Culture and SCLC Tissue Samples. SCLC cell lines NCI H69, H82, H209, H345, H378, H417, H510, H592 were generously provided by Phelps et al. and Carney et al. (28, 29). H82 cells were grown in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 5 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 100 units/ml penicillin, and 100 μg/ml streptomycin. These lines encompass both classic, neuroendocrine-differentiated SCLC cell lines (H69, H209, H345, H378, H417, H510, H592) and variant SCLC cell lines that lack neuroendocrine differentiation (H82, H417; Ref. 29). In addition, these lines have previously been reported to express nicotinic and/or muscarinic receptors (17, 18, 30). Paraffin-embedded bronchoscopic biopsies of SCLC were obtained from the Department of Pathology of the Oregon Health and Science University. Five-μm sections were cut, and 1 section was stained with H&E to confirm the diagnosis; then other sections were processed for immunohistochemistry as described below.
Immunohistochemistry and Immunocytochemistry. Paraffin sections of SCLC were processed for immunohistochemistry as described previously (31). Antibodies used were mouse anti-ChAT (mAB 305; Chemicon International, Inc.; 1:400), rabbit anti-VAChT (H-V005; Phoenix Pharmaceuticals), and rat anti-a7 nAChR (mAB 319; 1:250; Ref. 32). Immunohistochemistry for SCLC was performed using Vector ABC reagents and VIP as a chromogen (Vector Laboratories, Burlingame, CA) for ChAT and AEC for ChAT. VIP is a proprietary peroxidase substrate from Vector Laboratories that yields a purple color. Intensity of immunohistochemical staining was scored from 0 to 4+ by two independent readers (E.R.S. and H.S.S.) (where 0 = no staining; 1+ = focal weak staining; 2+ = focal strong staining or diffuse weak staining; 3+ = diffuse medium staining; and 4+ = diffuse strong staining). Fluorescent immunohistochemistry on H82 cells was performed using Texas-Red conjugated secondary antibody.

RT-PCR. RT-PCR and Southern hybridization were used to investigate the expression of nAChR, mAChR, ChAT, VACHT, and CHT1 genes in SCLC cell lines. For nAChR, α3, α5, α7, β2, and β4 subunits were examined. For mAChR, M3 and M5 subtypes were examined. RNA was isolated from SCLC cell lines with Tri Reagent (Molecular Research Center, Cincinnati, OH). RT-PCR, gel electrophoresis and hybridization were performed as described previously (31). Primers for PCR and internal primers for probes that were used are shown in Table 1.

Real-Time PCR Analysis. Real-time PCR was used to quantify ChAT, VACHT, and α7 and β2 nAChR receptor subunit mRNA levels in SCLC cell lines H82, H69, H345, H378, H417, and H592. Procedures were as described previously (33) and primers for 18S RNA were used for standardization. The amount of ACh needed to achieve a 3:1 signal:noise ratio, was 30 fmol per sample was assayed at least in duplicate. The detection limit, defined as the amount of ACh needed to achieve a 3:1 signal:noise ratio, was 30 fmol per 20-μl injection.

Cell Proliferation Assay. H82 cells were used to evaluate the effects of endogenous ACh synthesis on cell proliferation because real-time PCR showed they had the highest levels of ACh of the SCLC cell lines tested. Drugs tested were the nAChR antagonist mecamylamine at 10^-7, 10^-6, and 10^-5 M (Sigma, St. Louis, MO) to inhibit ACh degradation. Drugs tested were the VACHT inhibitor (±)-vesamigol (Sigma), the choline transport inhibitor, hemicholinium-3 (ICN Biomedicals Inc., Aurora, OH) for 24 h. After incubation, cell suspensions were transferred to 15-ml centrifuge tubes and centrifuged at 1000 rpm for 2 min at 4°C. Supernatants were aliquoted, rapidly frozen on dry ice, and stored at -80°C. For ACh assay, supernatants were thawed, and 20 μl of supernatant was injected directly into the HPLC for enzyme-coupled electrochemical detection as described previously (34). Each sample was assayed at least in duplicate. The detection limit, defined as the amount of ACh needed to achieve a 3:1 signal:noise ratio, was 30 fmol per 20-μl injection.

Table 1  Primers used for RT-PCR

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ACh IS AN AUTOCRINE GROWTH FACTOR FOR SCLC
ACh IS AN AUTOCRINE GROWTH FACTOR FOR SCLC

RESULTS

Identification of ChAT, VACHT, and ChT1 Gene Expression in SCLC Cell Lines. If a cholinergic autocrine loop is functional in SCLC, then proteins to synthesize ACh must be present and cells must express receptors for ACh. To determine whether SCLC cell lines express the proteins needed to synthesize ACh, ChAT, VACHT, and ChT1 gene expression was examined by RT-PCR in SCLC cell lines H69, H82, H345, H378, H417, and H592. All of the tested SCLC cell lines expressed ChAT and VACHT although only H345 cells expressed ChT1 (Fig. 1A). In the brain, multiple forms of ChAT are expressed. As shown in Fig. 1B, mRNA coding for three ChAT isoforms, N, R, and S, was expressed in all of the SCLC cell lines. ChAT isoform M was not expressed in any of the SCLC cell lines (data not shown).

Sequence Analysis of H82 Cell ChAT. To determine whether the ChAT mRNA expressed in SCLC was similar to neuronal ChAT, cDNAs spanning the entire coding region of ChAT were amplified, subcloned, and sequenced from H82 mRNA. This was done in two overlapping amplifications spanning exons 5–11 (Fig. 2A) and 11–18 (Fig. 2B) based on the genomic structure of ChAT as described by Ohno et al. (24). As shown in Fig. 2A, primers from exon 5–11 amplified 2 bands. The predominant band corresponded exactly to the size predicted from the sequence of neuronal ChAT. A second smaller band was also amplified. Sequence analysis of the amplified bands showed that the sequence of the larger band was identical to that of neuronal ChAT, whereas the smaller band corresponded to neuronal ChAT missing exon 10. As shown in Fig. 2B, the primers from exon 11 to exon 18 amplified a single band that corresponded to the size predicted from neuronal ChAT. Sequence analysis of this band showed it to be identical to neuronal ChAT. Thus the ChAT protein expressed by H82 cells is identical in sequence to the ChAT expressed in neuronal tissue.

Expression of mACHR and nACHR in SCLC Cell Lines. All of the SCLC cell lines except H378 expressed the α7, α5, and β2 nACHR subunit mRNAs (Fig. 3A). α3 nACHR was expressed in SCLC cell lines H69, H82, H345, H417, H592, was absent in H378, and was not tested in H209 and H510. β4 nACHR was expressed in SCLC cell lines H69, H82, H209, and H345, but not in H378, H417, H510, and H592. The M3 mAChR and M5 mAChR were expressed in all of the SCLC cell lines tested (Fig. 3B).

Quantitative Analysis of ChAT, VACHT, α7, and β2 nACHR Subunit mRNA. To quantify levels of mRNA expression and to further confirm expression, real-time PCR was performed for selected cholinergic mRNAs. As shown in Table 2, real-time PCR with different primer sets confirmed the expression observed by conventional RT-PCR. The quantitation achievable with real-time PCR also showed that there was significant variation in levels of ChAT, VACHT, α7 nACHR, and β2 nACHR mRNA among the SCLC cell lines tested. Relative levels of ChAT mRNA in cell lines tested were as follows: H82>H69>H417>H345>H378>H592. ChAT mRNA was highest in H82 cells and more than 70 times greater than the lowest, H592, cells. Relative levels of VACHT were H417>H345>H82>H592>H69>H378. α7 nACHR subunit mRNA levels were H82>H345>H592>H417>H69>H378, and β2 nACHR subunit mRNA levels were H592>H345>H82>H417>H69>H378. ACh Secretion by H82 Cells. Synthesis and secretion of ACh by H82 cells was confirmed by HPLC with enzymatically coupled elec-

Fig. 1. Expression of ChAT, VACHT, ChT1 mRNA in SCLC cell lines. RT-PCR and Southern hybridizations were performed on total RNA prepared from the indicated cell lines. Primers and probes are described in “Materials and Methods.” A: ChAT, VACHT, and ChT1 expression in SCLC cell lines H69, H82, H345, H378, H417, and H592. Human basal forebrain RNA was used as a positive control (HBBP43, HBBP45). B: ChAT isoform gene expression. ChAT N, S, R isoform genes were expressed in all of the SCLC cell lines tested. ChAT M isoform was not expressed in any of the SCLC cell lines (data not shown).

Fig. 2. PCR amplification of ChAT from H82 RNA. Upper panel, strategy used to amplify the ChAT mRNA. The entire coding region was amplified in two overlapping segments, A and B: segment A, the amplification of exons 5–11; segment B, the amplification of exons 11–18. UT, untranslated. A, RT-PCR from exon 5 to exon 11. B, RT-PCR from exon 11 to 18. In A, the 987-bp band is exactly the predicted size based on neuronal ChAT. The 810-bp band corresponds to authentic ChAT missing exon 10. In B, the 1151-bp band is exactly the predicted size based on neuronal ChAT. The identity of the amplified bands was confirmed by subcloning and sequence analysis as described in “Materials and Methods.”

10–5 M; the mAChR antagonist atropine at 10–6, 10–7, and 10–8 M; nicotine at 10–6, 10–7, 10–8, and 10–9 M; carbahol, a stable ACh analogue, at 10–6 and 10–7 M; the VACHT inhibitor vesamicol at 10–7, 10–8, and 10–9 M; and the choline transport inhibitor hemicholinium-3 at 10–6, 10–7, 10–8, and 10–9 M. All of the experiments for each drug were performed at least twice with a minimum of 12 replicates per data point per experiment. All of the drugs were from Sigma. H82 cells were plated with an 8-channel pipette at 5000 cells/well in 96-well plates. Drugs were added immediately after cell plating. The final medium volume of each well was 200 μL. Every 3 days, one-half of the volume of the media and drugs were changed after centrifugation of the plates at 1000 rpm for 1 min. At 0, 6, 9, and 12 days of incubation, a MTS-based assay was performed on the media and drugs were changed after centrifugation of the plates at 1000 rpm for 1 min. At 0, 6, 9, and 12 days of incubation, a MTS-based assay was used to measure cell growth.
trochemical detection. As shown in Fig. 4, A and B, ACh was easily detected in supernatants of H82 cell cultures. No ACh was detected in the media prior to cell culture. Addition of $5 \times 10^{-5}$ M neostigmine significantly increased media ACh levels ($P < 0.001$). This confirms the synthesis of ACh by H82 cells and also suggests the presence of acetylcholinesterase activity in the H82-conditioned culture medium. ACh was also readily detectable in supernatants of H345 cell cultures, thus demonstrating that ACh is secreted by both classic and variant SCLC cell lines. Next, vesamicol, an inhibitor of VAcHT, was used to determine the role of VAcHT in ACh release from H82 cells. As shown in Fig. 2C, vesamicol reduced H82 cell ACh basal release in a concentration-dependent manner. Vesamicol ($10^{-6}$ and $10^{-5}$ M) significantly decreased H82 cell ACh release ($P = 0.012$ and $P = 0.013$, respectively), suggesting the presence of a vesamicol-sensitive VAcHT activity in H82 cells. However, the ACh decrease caused by $10^{-5}$ M vesamicol was only 40%, suggesting that a vesamicol-independent pathway for H82 ACh release may also exist in SCLC cells. Next, the sensitivity of ACh secretion to hemicholinium-3 was determined. As shown in Fig. 4D, hemicholinium-3 reduced H82 cell basal ACh release in a concentration-dependent manner. Concentrations of $10^{-7}$, $10^{-6}$, and $10^{-5}$ M hemicholinium-3 resulted in the significant decrease of H82-cell basal ACh release in the presence of $5 \times 10^{-5}$ M neostigmine ($P < 0.010$, $P < 0.047$, and $P < 0.037$, respectively), suggesting the presence of a hemicholinium-3-sensitive, choline transporter in H82 cells.

**Regulation of H82 Cell Growth.** The role of endogenously synthesized ACh in regulating H82 cell growth was determined with inhibitors of ACh synthesis and with cholinergic receptor antagonists. As shown in Fig. 5A, the nAChR antagonist mecamylamine inhibited H82 cell growth in a concentration-dependent and time-dependent manner ($P < 0.05$). A concentration of $10^{-5}$ M mecamylamine significantly inhibited H82 cell growth at 6, 9, and 12 days ($P < 0.05$). A concentration of $10^{-6}$ M mecamylamine significantly inhibited H82 cell growth only at 6 days ($P < 0.05$). As shown in Fig. 5B, the mAChR antagonist atropine also inhibited H82 cell growth in a concentration-dependent and time-dependent manner ($P < 0.05$). A concentration of $10^{-6}$ M atropine significantly inhibited H82 cell growth at 6, 9, and 12 days ($P < 0.05$). As shown in Fig. 5C, the VAcHT inhibitor vesamicol decreased H82 cell growth in a concentration-dependent and time-dependent manner ($P < 0.05$). Concentration of $10^{-7}$, $10^{-6}$, and $10^{-5}$ M vesamicol significantly inhibited H82 cell growth at 9 and 12 days ($P < 0.05$). Similarly, the choline transport inhibitor hemicholinium-3 inhibited cell proliferation in a time- and dose-dependent manner (Fig. 5D). Showing that these were not just nonspecific toxic effects, the nicotinic agonist nicotine and the muscarinic agonist carbachol significantly stimulated cell growth in time- and dose-dependent manners (Fig. 5, E and F).

**ChAT Expression in SCLC Tumor Biopsies.** Immunohistochemistry was performed to determine whether immunoreactive ChAT could be detected in paraffin sections of SCLC biopsies. As shown in Fig. 6, ChAT could be readily detected in SCLC. In 14 of 26 SCLC tumor biopsies tested, ChAT immunoreactivity was detected (Fig. 6. A–C) with average immunostaining intensity of $2+$. As a positive control, a7 nAChR was tested and found to be expressed in 24 of 26 SCLC tested with average immunostaining intensity of $2+$. All of the tumors that expressed ChAT also expressed a7 nAChR, although there was no significant correlation between the intensity of ChAT immunostaining and a7 immunostaining. ChAT immunoreactivity was also detected in H82 cells (Fig. 6D). H82 cells and tumor samples also expressed VAcHT immunoreactivity (data not shown). Thus, these data demonstrate that SCLC tumors as well as SCLC cell lines express ChAT.

**DISCUSSION.**

This study presents data that SCLC express a cholinergic autocrine loop that can regulate cell growth. The data presented here demonstrate that: 1, genes for all components of an ACh autocrine loop including ChAT, VAcHT, CHT1, nAChR and mAChR are expressed in SCLC cells; 2, ChAT immunostaining is present in biopsies of SCLC and in SCLC cell lines; 3, SCLC cells are able to synthesize, secrete, and degrade ACh; and 4, SCLC cell growth is modulated by endogenous ACh synthesis. To our knowledge, this is the first study to demonstrate that SCLC cells have a cholinergic phenotype and that ACh exerts as an autocrine growth factor in human lung tumors.

For SCLC to express a cholinergic autocrine loop, ACh must be synthesized and secreted. The enzyme ChAT is required for ACh synthesis and, therefore, the expression of ChAT in SCLC must be definitively demonstrated to prove the existence of the autocrine loop. Because of its importance, we have, therefore, demonstrated the expression of ChAT in SCLC by multiple techniques. The presence of ChAT mRNA has been demonstrated by both conventional and real-time PCR with multiple independent primer sets. SCLC ChAT has been amplified and sequenced to show that the mRNA is authentic ChAT. The presence of ChAT protein has been demonstrated by

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Table 2. ChAT, VAcHT, a7- and b2-mAChR subunit mRNA levels in SCLC cell lines

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<th>b2-mAChR</th>
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<td>H69</td>
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<td>H417</td>
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Fig. 4. ACh release from H82 cells. H82 cells were plated as described in “Materials and Methods.” Drugs were added immediately after plating and cells incubated for 24 h; then media were removed for HPLC assay of ACh. A, HPLC chromatogram from cells cultured in the absence of neostigmine. B, HPLC chromatogram from cells cultured in the presence of 5 × 10⁻⁶ M neostigmine. C, effect of vesamicol on ACh levels in medium from cultured H82 cells. Drugs were added immediately after plating at the concentration shown and media was removed for assay 24 h later. Neostigmine (Neo) concentration = 5 × 10⁻⁶ M (n = 6). D, effect of hemicholinium-3 on ACh levels in medium from cultured H82 cells. Drugs were added immediately after plating at the concentration shown and medium was removed for assay 24 h later. Neostigmine (Neo) concentration = 5 × 10⁻⁶ M (n = 6). Data are represented as mean ± SE. †, P < 0.05 for no neostigmine compared with 5 × 10⁻⁷ neostigmine. ∗, P < 0.05 compared with no vesamicol (Ves) or hemicholinium-3 (Hem-3) but with neostigmine.
dependent manner, VAChT appears to be active in SCLC cells. This is further supported by the immunohistochemical detection of VAChT in SCLCs (not shown). On the other hand, in the H82 cells, 10^{-5} \text{M} vesamicol decreased ACh release by only 40% as opposed to the larger suppressions seen in the neuronal cells (44). This suggests that SCLC cell lines may also use a second, vesamicol-insensitive, perhaps nonvesicular, mechanism for ACh secretion as has been reported for placenta (46).

For a cholinergic autocrine loop to exist, SCLC must also express receptors for ACh. There are two classes of ACh receptors, nAChR and mAChR. mAChR are G protein-coupled receptors. nAChR consist of five homologous or heterologous subunits. To date, cDNA sequences for 17 kinds of nAChR subunits have been determined (47). These include \( \alpha_4-\alpha_{10}, \beta_1-\beta_4, \gamma, \delta, \) and \( \epsilon \) (47). In the central nervous system, the most abundant heteromeric form is \((\alpha_4)_2(\beta_2)_2\), the most abundant homomeric form is \((\alpha_7)_5\). Autonomic ganglia express a complex mixture of \( \alpha_3, \alpha_5, \alpha_6, \beta_2, \) and \( \beta_4 \) receptors. As shown in Fig. 3, SCLCs express \( \alpha_3, \alpha_5, \alpha_7, \beta_2, \) and \( \beta_4 \) nAChR. This pattern is consistent with the expression of \((\alpha_7)_5\) homomers and ganglionic heteromeric forms of nAChR in SCLCs. Also, as shown in Fig. 3, all of the SCLC cell lines tested expressed the M3 and M5 mAChR.

Our identification of nAChR in SCLC cell lines and tumors is consistent with other reports of nAChR in SCLCs. The initial observation of the effects of nicotine on SCLCs dates to Lauweryns et al. (48), who noted that nicotine caused degranulation of rabbit pulmonary neuroendocrine cells. Subsequently Cunningham et al. (16) showed the presence of both nicotinic and muscarinic receptors on SCLCs. Chini et al. (30) and Tarroni et al. (15) did the first detailed description of subtypes of nAChRs in SCLC and reported the presence of \( \alpha_3, \alpha_5, \beta_4, \) and \( \alpha_7 \) nicotinic subunits; and Quik et al. (18), similarly, reported the presence of \( \alpha_7 \) nAChR in SCLC. Thus the nAChR subtypes that we have detected are consistent with previous reports. Similarly, our observation of M3 receptors in SCLC cell lines is also consistent with other reports (14, 20, 49).

Although the components for a cholinergic autocrine loop are clearly present in SCLC, to prove an autocrine loop exists, modulation of growth must be demonstrated. To prove this, we have demonstrated autocrine stimulation at multiple levels in the cholinergic loop. The inhibition of choline transport with hemicholinium-3 decreased ACh synthesis and significantly decreased cell proliferation (Fig. 5). The inhibition of VAChT with vesamicol decreased ACh synthesis and significantly decreased cell proliferation. Blockade of the receptors for endogenously synthesized ACh with muscarinic and nicotinic...
The extent to which these variations in ChAT and cholinergic receptor expression is related to tumor growth, progression, or prognosis also requires additional studies. It is also clear that only a subset of SCLC will express this cholinergic autocrine loop and that some SCLC will not synthesize or respond to Ach and that some SCLC may only degrade Ach. Additional studies are required to determine how widespread the expression of the cholinergic autocrine loop by SCLC will be.

The expression of a cholinergic phenotype by SCLC may help explain the SCLC paraneoplastic syndrome, LEMS, which is characterized by antineuronal antibodies leading to myasthenia (56). It is possible that the presentation of cholinergic antigens such as ChAT, VACHt, and CHT1 results in production of antibodies targeted against tumor cells that react with central and peripheral cholinergic nerves and lead to LEMS. The expression of splice variants of ChAT, as shown in Fig. 2, may also promote autoantibody production. Given that antibodies against neuronal nAChRs have been reported in SCLC patients with paraneoplastic neurological syndromes (57), it is likely that antibodies to these other cholinergic factors can be produced. Interestingly, in a recent report, SCLC patients with LEMS had a significantly longer survival compared with SCLC patients without LEMS (58), which suggests that antibodies to cholinergic proteins may impair tumor growth.

In summary, our data demonstrate the expression of a cholinergic autocrine loop in SCLC and provide a number of new targets for modifying tumor growth. These findings also provide a theoretical basis for understanding the basis by which nicotine and related compounds modulate lung cancer growth.

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ACH IS AN AUTOCRINE GROWTH FACTOR FOR SCLC


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