The focus on growth factors as a target for therapeutic control of malignant proliferation arose from the early observations of Barnes and Sato\(^1\) and others regarding the fundamental role of peptide hormones in permitting the \textit{in vitro} propagation of tumor cells. The possibility that tumor cells could mediate their own growth stimulation (autocrine growth) further intensified interest in growth factor dynamics.\(^1\) In the area of lung cancer 4 autocrine growth factors have been described, as summarized in Table 1. Gastrin-releasing peptide (GRP) was the first autocrine factor identified with activity in lung cancer. That biology is the basis for a phase I/II clinical trial to determine if a monoclonal antibody (MoAb) with high affinity and specificity for the biologically active region of GRP can be employed to disrupt the autocrine-mediated proliferation of small cell lung cancer.\(^4\) In this review we consider aspects of the biology of autocrine growth stimulation as relevant to a therapeutic perspective. Although recent work has served to extend vastly the complexity of these mechanisms, systematic evaluation of therapeutic strategies has a reasonable probability of eventual control of malignant proliferation.

The outcome of the initial anti-GRP MoAb trial in patients with small cell lung cancer will hinge on two critical issues. The first is whether a molar excess of the monoclonal antibody can be delivered throughout all proliferating compartments of the tumor. This can be measured indirectly by measuring the circulating serum level of the antibody or a marker of GRP effect, serum gastrin. The only direct measure of antibody penetration will be to evaluate tumor biopsy specimens during antibody therapy to determine the degree of saturation of antibody binding to secreted peptide. The second and more fundamental issue is whether antibody-mediated sequestration of the autocrine growth GRP is sufficient to effect clinically meaningful cytostasis or cytotoxicity. This final issue is especially topical in light of the other recently described autocrine growth factors summarized in Table 1.

Currently one can only speculate about the interrelationships of the various autocrine growth factors and many other questions are unanswered. How many more molecules capable of mediating autocrine effects exist? Are these molecules interacting as part of a cascade? Can certain growth factors substitute for one another through a particular class of receptors? Are tumor-related growth factors sufficiently distinctive from factors required for normal homeostasis to permit a host to survive acute interruption of one or more of these autocrine loops? Do tumor-related growth factors mediate their growth effects through the same or different signal transduction pathways as normal processes? If antioncogenes, as recently described in the situation of the retinoblastoma gene, do exist as a general mechanism,\(^5\) do they operate on the level of the growth factor to control neoplastic proliferation?

This daunting litany of questions molds the directions currently employed by investigators now working in this field. Although the inherent correctness of one's research direction will only be established retrospectively, the remainder of this review will deal with the directions used here at the NCI-Navy Medical Oncology Branch in attempting to exploit growth factor biology in the therapy of human
Table 1—Autocrine Growth Factors in Lung Cancer

<table>
<thead>
<tr>
<th>Factor</th>
<th>Histology</th>
<th>Inhibitor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrin releasing peptide*</td>
<td>SCLC</td>
<td>aGRP MoAb</td>
<td>3, 4, 23, 24</td>
</tr>
<tr>
<td>Insulin like growth factors 1*</td>
<td>SCLC</td>
<td>αIGF-I receptor MoAb</td>
<td>10, 12, 14, 25, 26</td>
</tr>
<tr>
<td></td>
<td>NSCLC</td>
<td>atransferrin receptor MoAb</td>
<td>13, 15</td>
</tr>
<tr>
<td>Transferrin*</td>
<td>SCLC</td>
<td>aEGF receptor MoAb</td>
<td>16</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>SCLC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(transforming growth factor α)</td>
<td>NSCLC</td>
<td></td>
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</tbody>
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*Actual tumor product may be immunologically cross reactive but structurally distinct from the parent molecule.

lungs cancer.

Following the observation of Sato, Simms and co-workers established a serum-free, hormonally defined media formulation that supported the in vitro propagation of small cell lung cancer cell lines. The formulation included hydrocortisone, insulin, transferrin, β-estradiol, and selenium in RPMI 1640 basal media (HITES). This formulation was used by Carney and co-workers in the experiments establishing the role of amphibian bombesin (or its mammalian counterpart, GRP) as a mitogen for small cell lung cancer. This mitogenic activity of GRP is masked in serum-containing media formulations, highlighting the utility of a serum-free formulation. In that experience, the two most potent mitogens in the HITES media were transferrin and insulin, respectively.

Cuttitta et al in a series of rabbit inoculations with conditioned media from representative small cell lung cancer cell lines attempted to use the rabbit immune response to determine the most significant constituents of the conditioned media. In solid phase radiobinding assays the peptide hormones most avidly recognized were transferrin and insulin. These 2 lines of evidence suggested the possibility that insulin and transferrin are autocrine growth factors for small cell lung cancer.

In considering insulin as a small cell mitogen, it was significant that Gazdar and co-workers had found that less than 10% of small cell lung cancer lines produced insulin. The Cuttitta group's rabbit immunization experiments pointed not to insulin but more accurately to a molecule immunologically cross-reactive to insulin. These facts led us to explore the mitogenic effect of insulin-like growth factor-1 (IGF-I). As recently reported, growth curves of IGF-I and insulin showed IGF-I to be equipotent with insulin with regard to maximal growth stimulation, but this maximally stimulatory effect required a concentration of IGF-I 1-2 logs less than insulin. Van Wyk first noted this phenomenon in the situation of insulin acting as a mitogen via the IGF-I receptor. Radiolabeled receptor binding studies confirmed that small cell lung cancer expressed specific, high-affinity IGF-I receptors similar to those previously described on human placental cells. The small cell lung cancer cell lines expressed a peptide molecule detected on western blot that was immunologically consistent with a pro-IGF-I molecule. Finally, using a neutralizing MoAb specific for the IGF-I receptor, small cell lung cancer cell lines grown in hormonally-defined, insulin, and IGF-I free media were inhibited approximately 25%. Since this decrement is small, multiple repeats were performed which demonstrated reproducibility of this finding. Natale et al, while with our group, reported that non-small cell lung cancer cell lines also frequently express an IGF-I precursor molecule and high-affinity IGF-I receptors. Parallel anti-IGF-I receptor MoAb experiments as previously described also conducted in the absence of exogenous, insulin or IGF-I showed a 20% decrease in non-small cell growth.

The second suspected mitogen, transferrin, appears to be somewhat more potent in growth studies. Both peak stimulation with exogenous transferrin and blockage of intrinsic transferrin effect by an antitransferrin receptor MoAb appear to be of greater magnitude than the IGF-I effects. Nevertheless, only approximately 50% of small cell line growth is inhibited by the antitransferrin receptor MoAb. In preliminary in vitro experiments to date, combinations of anti-GRP MoAb, the anti-IGF-I receptor MoAb, plus the antitransferrin receptor MoAb, have effects only modestly greater than the anti-transferrin receptor MoAb (which is the most potent in this assay system). This raises the possibility that additional autocrine factors are being produced by small cell tumors.

Epidermal growth factor (EGF) is the final molecule reported to mediate autocrine stimulation for lung cancer. Autocrine growth factor specifically for the squamous cell type of non-small cell lung cancer is the focus of a trial with eventual clinical intent at Memorial Sloan Kettering. The initial phase of this study is the use of an radioconjugate of an anti-EGF receptor antibody to determine its pharmacokinetics and its efficiency in tumor localization. EGF does not appear to have mitogenic activity for small cell lung cancer.

To search for additional peptide hormones being produced by small cell lung cancer which might have growth effects, we have developed a new in vitro approach. We speculated that the best way to determine the full self-stimulatory capability of a cell line was to grow a tumor cell line in the complete absence of any hormonal supplementation. The preliminary experience with this approach was recently described. Conditioned media from such cell lines which has been shown to be growth stimulatory would be a potentially useful substrate in evaluating for the presence of other new autocrine growth factors. Historically this analysis has been limited to factors for which immunologic reagents (either hetero-antisera or specific MoAb) existed. We have recently reported an approach using HPLC integrated with mass spectrometry, which potentially circumvents this limitation. Many peptide hormones share a structural feature of carboxy terminal amidation. This feature has previously been exploited by Tatemoto and Mutt as a signature of biologic activity. The mass spectrometry approach can simplify this process and brings us closer to the goal of on-line resolution of tumor cell peptide secretion.
Whether knowledge of peptide hormone secretion can be of benefit in detecting as well as treating lung cancer is highly speculative. Recent developments suggest one direction. In 1987, Aguayo and co-workers hypothesized that the bronchial injury caused by smoking caused an increased release in bronchial neuropeptides involved in wound repair, such as GRP, and that this long-term exposure may be pathogenically related to the ultimate development of lung cancer. This observation was consistent with our group's working hypothesis of growth factor effects promoting the very earliest stages of lung cancer and dovetailed with another of our research efforts. In collaboration with a group from Johns Hopkins that conducted the Early Lung Cancer Detection study sponsored by the NCI during the 1970s, we analyzed if changes in cell surface antigen composition of expectorated bronchial cells could identify those patients with moderate to severe bronchial dysplasia at high risk for developing lung cancer. This analysis was performed by staining sputum cells archived from the Early Detection study on those 15% of all lung cancer patients who were found to have moderate to severe cytologic dysplasia. Two monoclonal antibodies, one derived against small cell and the other against non-small cell, were used for the staining. This procedure with a 90% accuracy identified patients who developed lung cancer over the following 2 years. With 70% accuracy this same analysis identified patients 4 years before the development of clinically detectable lung cancer.

We speculate that with this time frame it is possible to detect lung cancer when the cancer is still confined to the dysplastic bronchial mucosa. At this point in the cancer's natural history, there is a chance that local modalities may be curative. Among the strategies that one could envision applying in the setting of earlier detection is a specific biochemical disruption of the appropriate autocrine growth factors. If one views lung carcinogenesis as a result of clonal expansion, anti-growth factor strategies may be uniquely suited for successful anti-cancer intervention approaches. Concerted efforts to evaluate such approaches, although highly speculative, appear as reasonable as, or more than, any other options in dealing with this most frustrating and lethal of all cancers.

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The Clinical Relevance of Recent Developments in Pathology and Biology of Small Cell Lung Cancer*

Fred R. Hirsch, M.D.; and Heine H. Hansen, M.D., F.C.C.P.

Small cell lung cancer (SCLC) has emerged over the last 10-15 years as a distinct pathologic and clinical entity, characterized by early and wide dissemination and with a great sensitivity to both chemotherapy and radiotherapy. Despite the objective response to chemotherapy in more than 85%, most of the patients sooner or later develop clinical relapse, and fewer than 10% become long-term survivors. The need is thus urgent for more biologic knowledge about this disease, and great efforts have been put into this field of research by scientists all over the world. Through detailed studies, especially on more than 100 established cell lines from SCLC, much biologic information has been accumulated including the relationship of SCLC to other histologic types of lung cancers and to endocrine tumors. The present short overview deals with the clinical significance of recent scientific developments in the histopathologic and biologic studies of SCLC.

Histopathologic Classification of SCLC

The World Health Organization (WHO) published the first international classification of malignant lung tumors in 1967, with a subsequent revision in 1981. In the last revision SCLC was subdivided into 3 morphologic subtypes: “oat-cell type,” “intermediate,” and “combined oat-cell,” the last includes SCLC in combination with squamous cell and adenocarcinomas. The criteria for the histopathologic classification have been discussed in detail previously. The recognition of some SCLC tumors having features of large cell carcinoma was made by the WHO panel of pathologists, and these tumors were included in the “intermediate” category.

Since the last WHO revision much attention has been given in clinical-pathologic studies to the search for a prognostic significance of the WHO classification of SCLC. Two large independent studies from the National Cancer Institute, USA, and the Finsen Institute, Denmark, failed to demonstrate any difference in response and survival to chemotherapy comparing patients with “oat-cell” and “intermediate” subtypes. However, patients having SCLC with some large cell features showed a significantly lower response rate and shorter survival than those with pure SCLC. These studies subsequently led to a modification of the subclassification by the International Association for the Study of Lung Cancer (IASLC), in which all pure SCLC tumors were put into the same category, while SCLC with large cell features was separated as a special subgroup. More recently, a preliminary report was published by the Eastern Cooperative Group in USA, in which the IASLC classification was applied and no clinical difference between the pure SCLC and SCLC/large cell tumors was found for SCLC patients with “extensive disease.” The differences in results between the studies might reflect interobserver variability problems in the morphologic classification, which ought to be solved prospectively in future studies.

Comparing pretreatment and posttreatment histopathologic material, a considerable change in morphology has been observed. The clinical relevance of the morphologic variations was studied by 2 investigators. In both studies patients with mixed histology after treatment had a significantly shorter survival than those with pure SCLC morphology. Whether these morphologic variations were pre-existing or due to the treatment with cytostatic drugs is still uncertain.

Immunohistochemistry

It has been known for decades that SCLC has a great potential for producing multiple endocrine “markers.” Following the rapid development in immunocytochemistry and monoclonal antibodies during the past decade, much interest has been devoted to a variety of malignant lung tumors including SCLC.

The studies have particularly concentrated on the following subjects: (A) general neuroendocrine markers, (B) specific peptides, and (C) more unspecific small cell antigens detected by monoclonal antibodies.

The purpose of the study has been to achieve more information about the biology of the tumors, the interrelationship between the different histologic types, and to reveal whether various antibodies can be used to improve the histopathologic classification.

Immunohistochemical Characteristics of SCLC and their Relationship to Other Malignant Lung Tumors

Various studies using continuous cell lines have revealed that SCLC produces a number of hormones and hormone-like substances, which can be demonstrated by “traditional” immunocytochemical techniques such as immunoperoxidase staining.

A. Common neuroendocrine markers: Among the most interesting neuroendocrine markers studied by immunohistochemistry in SCLC are: neuron-specific enolase (NSE), chromogranin A, neurotensin, synaptophysin, and protein gene product 9,5 (Pgp 9,5).

In order to find a specific diagnostic marker considerable interest has been attached to NSE. While most studies have identified NSE in almost all SCLC-tumors (70-100%), several studies have also demonstrated NSE in a variable fraction of non-SCLC tumors (15-55%).

Chromogranin was suggested to be a promising neuroendocrine marker by Wilson and Lloyd, and it was demonstrated in 4/10 SCLC tumors. Others have not been able to reproduce the high frequency of expression of chromogranin in SCLC. In a more recent study by Hirsch et al., chromogranin was demonstrated in 25% of the SCLC tumors and in 5% of non-SCLC tumors.

Another recently characterized neuroendocrine marker in SCLC is synaptophysin, which has been identified in 79% of 68 SCLC tumors, compared with 8% of 74 non-SCLC tumors. Future studies have to verify whether this is a useful SCLC-marker in the diagnosis of malignant lung tumors.

A new antigen originally extracted from the brain and

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