Recent Advances in the Biology of Small Cell Lung Cancer

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Advances in the techniques for culturing human tumors in vitro, especially lung cancer cells, have greatly facilitated studies of the biologic properties of both small cell and non-small cell lung cancer cells. Detailed analysis has been done of well-characterized cell lines of both groups with respect to growth properties, biomarker and antigen expression, cyto- genetics, and oncogene amplification and expression. Two major conclusions have emerged from these studies: (1) considerable heterogeneity exists within a given tumor type (eg, SCLC) in the expression of a given biomarker, and (2) overlap in the expression of biomarkers exists between cells of SCLC and non-SCLC, suggesting a common stem cell for all types lung cancer. In the future, clinical trials the impact of the biologic properties of cells on responses to therapy and survival will need assessment.

In 1985 approximately 135,000 new cases of lung cancer will have been diagnosed in the United States, and approximately 110,000 persons will have died of this disease.1 In spite of major advances in the diagnosis staging and treatment of lung cancer, overall results and survival for new patients has remained essentially unchanged over the past 20 years. Small cell lung cancer (SCLC) accounts for 20-25% of new cases of primary lung cancer and differs clinically and biologically from the other histologic types of lung cancer collectively referred to as non-SCLC (NSCLC) lung cancers. While surgical resection offers the only hope for long-term survival in patients with NSCLC, chemotherapy with or without radiation therapy offers the best hope for survival in SCLC patients.4

In detailed chemotherapeutic studies of patients with SCLC and NSCLC, it is clear that considerable heterogeneity in therapeutic responses are observed between SCLC and NSCLC and within a given histologic subtype (ie, SCLC) of these tumors. Results from many studies have demonstrated that up to 90% of patients with SCLC, and perhaps 20-40% of patients with NSCLC, achieve a clinical response to cytotoxic therapy. While many factors may play a role in responses such as performance status or disease extent, it is also possible that properties inherent within the cells themselves may account for differences in therapeutic responses. If biologic properties or markers could be identified which correlate with either a response or lack of response to therapy, these markers would have an important impact on both therapy selection and survival.

In recent years many laboratories have reported on the establishment and characterization of cell lines of both SCLC and NSCLC. Results from these studies have confirmed the heterogeneity that exists within a given tumor type and have demonstrated that more overlap exists in the biologic properties between SCLC and NSCLC that has been previously realized. The biologic characteristics of SCLC tumors will be described and compared with those of NSCLC tumors. The potential application of these properties in the management of patients with lung cancer will be outlined.

CELL LINES OF SMALL CELL LUNG CANCER

Several recent publications have described in detail methods for the establishment of cell lines of SCLC.5-8 Initial studies using undefined serum supplemented medium (SSM) were associated with success rates in establishing lines of only 10%.5 In recent years, using a chemically defined medium (HITES) with or without serum supplementation (2-5% fetal bovine serum), cell lines of SCLC have been reproducibly established from 75% of all tumor-containing biopsy specimens.6-8 Cell lines have been established from a variety of organ sites including lung, bone marrow, lymph nodes, malignant effusions, liver biopsies, and other surgically resected masses (brain, adrenals), etc. In general, the biologic properties of cell lines established from different sites are similar.8 The ability reproducibly to grow SCLC tumor cells in vitro from the majority of SCLC patients has greatly facilitated a systematic study of the biologic properties of these cells.

SCLC cell lines usually grow as tight to loosely packed aggregates of floating cells, unlike lines of NSCLC, which grow as attached monolayer cultures of cells. Once established as permanent cell lines, these cells can be successfully cryopreserved, will form colonies in soft agarose and tumors in athymic nude mice, and can be maintained indefinitely in cell culture.

BIOCHEMICAL MARKERS OF SMALL CELL LUNG CANCER: CLASSIC AND VARIANT PHENOTYPES OF SCLC

In a recent report 50 cell lines established from patients with SCLC were characterized with respect to their expression of 4 biomarkers including the key APUD enzyme L-dopa decarboxylase (DDC), the APUD enzyme neuron-specific enolase (NSE), the peptide hormone bombesin/gastrin-releasing peptide (BLI), and the BB isozyme of
may clearly represent an important biologic subset of SCLC tumors.

**Antigenic Expression in Small Cell Lung Cancer**

Monoclonal antibodies have potential widespread application in the diagnosis, treatment, and staging of lung cancer. Although many monoclonal antibodies (MoAbs) have been generated against lung cancer antigens, their broadscale use has been limited by the lack of relative specificity of individual antibodies and perhaps more importantly, the problem of antigenic and biologic heterogeneity in SCLC cells. While many of the reported MoAbs have potential uses in the evaluation of SCLC patients, several investigators have reported on the use of MoAbs both in the detection of SCLC cells in clinical specimens, and in the purging of contaminated bone marrow of tumor cells *ex vivo*. Using the MoAb SMI, an IgM MoAb generated using a SCLC cell line as an immunogen, Bernal et al demonstrated SCLC cells in 19 of 30 bone marrow specimens pathologically considered to be free of tumor, including eight of 16 specimens from patients otherwise felt to have limited stage disease. These data suggest that bone marrow metastases that cannot be detected by conventional histologic techniques can be detected using MoAb. In addition, if confirmed these data would have considerable impact in the management of patients with limited stage SCLC who are being considered for either surgical resection or autologous bone marrow transplantation.

Several MoAbs have recently been described which are cytotoxic to SCLC tumor cells *in vitro* and thus have the potential value in the *ex vivo* treatment of contaminated marrow in preparation for autologous reinfusion. In some studies up to 99.9% of SCLC tumor cells were lysed with MoAbs with or without complement. In addition the cytotoxic effect of the MoAbs is relatively specific for SCLC in that little or no effect was observed on either other forms of lung cancer cells or bone marrow stem cells. Thus, these antibodies provide for the future evaluation of *ex vivo* treatment of contaminated bone marrow in therapeutic clinical trials of SCLC.

Recently several investigators have reported that antigens, more frequently found in hematopoietic cells may be expressed in lung cancer cells. Using a panel of antibodies, Bunn et al found intense anti-leu 7 binding to the majority of cell lines and fresh specimens of SCLC while other lung cancer types were negative or showed only weak and focal binding. This antibody has previously been shown to react with natural killer (NK) cells. Other antigens expressed by NK cells, monocytes and lymphocytes were never or less often expressed on SCLC cells. The differential expression of the leu-7 antigen by SCLC and NSCLC may be of clinical value in the typing of lung cancer cells.

Another class of antigens evaluated in lung cancer cells include the class I major histocompatibility complex antigens (HLA-A,B,C) and B,Microglobulin. Recently Doyle et al demonstrated that these antigens are selectively expressed on lung cancer cells. HLA antigens and B,Microglobulin were not expressed, or expressed at very low levels in SCLC in contrast to NSCLC. Studies using molecularly cloned probes for HLA and B,Microglobulin showed that the genes for the antigens were
intact at the DNA level, but in most cases HLA and β₂M mRNA was not expressed or expressed at low levels. Incubation of SCLC cells with interferons lead to the development of detectable amounts of HLA and β₂M antigens on the cell surface. As these molecules play a role in immune cell recognition, the low or absent expression of HLA antigens in SCLC may account for its propensity for early widespread dissemination and rapid cell growth. The administration of agents that increase their expression may be of value in delaying or preventing tumor recurrence.

**INTERMEDIATE CELL FILAMENTS OF SMALL CELL LUNG CANCER**

Intermediate cell filament proteins (IFP) are tissue-specific proteins and represent an intracellular network of protein filaments of varying sizes. Five different types of IFP can be identified by an indirect immunofluorescence technique using specific antibodies directed against proteins that are part of this structure. It has also been demonstrated that tissue-specific IFPs are retained during neoplastic transformation of these tissues. Thus, antisera to IFP may be used to type the origin of neoplastic tissues. Several investigators have reported on the IFP characteristics of SCLC cell lines and fresh specimens and conflicting results have been noted. In some studies SCLC have been reported to contain neurofilaments, while in others cytokeratins and not neurofilaments have been identified. In studies in which the IFP characteristics and immunocytochemistry for neuroendocrine markers (e.g.,NSE) were evaluated on consecutive specimens, both cytokeratins and neuroendocrine markers were identified in individual cells. These data would suggest an epithelial origin for SCLC rather than a neuroendodermal origin for this tumor. As keratin proteins are also found in other forms of lung cancers, these data are consistent with the notion of a common stem cell for all lung cancers, both SCLC and non-SCLC.

**AUTOCRINE GROWTH FACTORS FOR SMALL CELL LUNG CANCER**

The ability of cancer cells to produce, secrete and respond to their own growth factors has become a central concept in studying mechanisms of growth regulation in human tumor cells. In studies of SCLC growth it has been demonstrated that these tumor cells could be established as cell lines, and maintained indefinitely in a serum-free defined medium at an efficiency greater than that observed in serum-supplemented medium. Studies using conditioned medium from established SCLC cell lines confirmed that SCLC cells secrete into this medium a mitogenic growth factor. More recently detailed studies of the peptide hormone bombesin have revealed that both bombesin and its related peptide, gastrin-releasing peptide (GRP), are potent mitogens for SCLC. In studies of both SCLC and NSCLC, cell line stimulation of clonal growth was only observed in SCLC lines. As previous studies of lung cancer cell lines have shown that bombesin/GRP is produced and secreted by SCLC lines, and that SCLC lines have high affinity binding receptors for BLI, these data strongly support the role for BLI/GRP as autocrine growth factors for SCLC. Confirmation of this hypothesis comes from the report that a monoclonal antibody 2A11, specific for the C-terminal portion of bombesin, not only inhibits the binding of bombesin to its receptors, but also inhibits both the in vitro clonal growth of SCLC cell lines and the in vivo growth of SCLC xenografts in athymic nude mice. The antibody had no effect on NSCLC lines. These data and others suggest that neuropeptides such as bombesin substance P, substance K, vasopressin, etc., may function as potent mitogens. It also appears that the mitogenic effects of these substances are associated in each instance with a specific subtype of tachykinin receptor. The identification and characterization of both peptides and their receptors offers the potential for a new form of endocrine treatment for SCLC and other human tumors.

**ONCOGENE EXPRESSION IN SMALL CELL LUNG CANCER**

Cytogenetic abnormalities have long been recognized in both established cell lines and fresh clinical specimens of SCLC. DNA content analysis by flow cytometry (FCM) has demonstrated considerable heterogeneity, with DNA contents ranging from hypodiploid to tetraploid in up to 85% of all tumor specimens. Multiple stem lines are present in 10-20% of specimens. However, unlike other tumors, no correlation has been observed between the DNA content and response to therapy and survival of patients with SCLC.

In many cell lines and fresh specimens a specific cytogenetic abnormality involving part or all of the short arm of chromosome 3 has been reported. While this deletion has not been observed in all specimens of SCLC, results of future studies will clarify its frequency and if its presence is associated with a specific subtype of SCLC which may be clinically important.

In addition to chromosome 3, numerical chromosomal abnormalities have been observed in almost all SCLC specimens. In several fresh specimens and cell lines, double minute chromosomes or homogeneous staining regions (HSRs) have been observed. While the presence of the latter abnormalities may in some instances be related to gene amplification and drug resistance, in other cases it appears that these changes are associated with amplification and increased expression of specific protooncogenes.

In detailed studies of 40 SCLC, cell lines amplification (20-70-fold) of the C-myc oncogene was detected in 8 cell lines. Seven of these cell lines belonged to the variant class of SCLC lines. These variant lines also expressed increased amounts of C-myc mRNA. In 4 C-myc amplified cell lines evaluated, one or more HSRs was noted in all cell lines, while none was detected in classic cultures. The association of C-myc amplification with the variant class of SCLC, which have a more aggressive growth behavior, suggests that this oncogene amplification may account for the more malignant behavior of these variant tumors. More recently amplification and increased expression of a C-myc related gene, N-myc, has been detected in several cell lines and fresh specimens of SCLC. These N-myc amplified cell lines belonged to both the classic and variant classes of SCLC, and double minute chromosomes have been detected in some of these cell lines. Other oncogene abnormalities including expression of the C-myb oncogene and activation of a ras...
oncogene have been detected in a small number of specimens of SCLC. Because of their presence in the minority of SCLC specimens, their significance remains uncertain. In contrast, the finding of amplification and/or increased expression of myc-related genes in almost 50% of cell lines of SCLC and their presence in fresh biopsy specimens clearly suggests that these genes play an important role in the establishment and/or malignant behavior of SCLC. Confirmation of these data is in the results by Johnson et al., who have shown that patients whose specimens or cell lines express these oncogene abnormalities have a significantly shorter survival time than these patients whose specimens lack these changes. Clearly, future clinical studies of SCLC need to correlate in a prospective fashion the expression of oncogenes with clinical responses and survival. Moreover, as the products of these genes and their function are identified it should be possible to target therapies in a more specific manner to alter the malignant behavior of these cells in vivo.

INTERRELATIONSHIP BETWEEN SMALL CELL AND NON-SMALL CELL LUNG CANCERS: ENDOCRINE AND NONENDOCRINE LUNG CANCER

In recent years it has become clear that considerable overlap exists between SCLC and non-SCLC. Histologic evaluation of specimens from lung cancer patients reveals more than one cell type in 10% or more of patients (eg, mixed small cell/adenocarcinoma). In autopsy studies of SCLC patients who died following systemic chemotherapy, mixed histologies were noted in 40% of patients, which suggests either the emergence of a second tumor or that individual lung tumors can undergo differentiation to other cell types (eg, small cell to squamous cell). Recent data suggest that a common stem may exist for all lung tumors, supporting the concept that individual tumors may spontaneously differentiate into tumors of other histologies. In studies of panels of cell lines of non-SCLC for the expression of endocrine biomarkers including DDC, elevated levels of these markers were detected in 15% of specimens. In addition, in studies of fresh NSCLC specimens, endocrine markers can be detected in a similar percentage of tumors. In the analysis of the surface protein phenotype of a cell line established from a patient with mixed SCLC and non-SCLC, the surface protein phenotype of the parent and clonal cell lines expressed characteristics of both SCLC and non-SCLC. Finally, among 52 cell lines established from patients with SCLC, 2 cell lines were noted spontaneously to undergo tripartite differentiation in vitro into SCLC, adenocarcinoma and squamous cell carcinoma (D. N. Carney, unpublished observation). This property of tripartite differentiation was retained by clonal cell lines. All of these data provide evidence for a common cellular origin of the different histologic types of lung cancer. The expression of endocrine properties by some but not all SCLC and NSCLC tumors suggests that in future clinical trials and in addition to routine histology, lung tumors should be classified by their expression of endocrine properties into endocrine and nonendocrine tumors to determine if the presence of these properties account for differences observed in responses to therapy and survival.

CONCLUSION

In recent years considerable insight has been gained into the biologic properties of both SCLC and NSCLC. By detailed studies of both fresh tumors, and more importantly established well-characterized cell lines biomarkers, such as endocrine properties or antigen expression that relate to the clinical behavior of a tumor, have been identified. The studies of SCLC clearly indicate that SCLC is not a single disease entity but a spectrum of diseases with different behavior and prognosis (ie, classic and variant SCLC). Moreover, we have recognized that lung tumors frequently exhibit multiple pathways of cellular differentiation. While the identification of biomarkers clearly associated with a single type of lung cancer would be of practical value, we have recognized that between the major subtypes of lung cancer (SCLC and NSCLC) there is overlap in the expression of markers.

In addition, in a single type of lung cancer (SCLC), heterogeneity of biomarker expression exists. The range of different biomarker expression within lung cancers suggests that in future clinical trials of lung cancer, clinical presentation, response to therapy, and survival must be correlated with biomarker expression. The results of such studies will indicate the importance of these biologic properties.

A recent paper by Nau et al. has described a third myc-related gene (L-myc) in cell lines and fresh biopsies of patients with small cell lung cancer. Gene mapping studies have assigned L-myc to human chromosome region lp32, a location distinct from that of either C-myc (chromosome 8) or N-myc (chromosome 2). Thus, in excess of 50% of SCLC cell lines studied myc-related genes have been identified, suggesting that such genes may be important in the growth and or transformation of small cell lung cancer.

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