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NCI Series of Cell Lines: An Historical Perspective

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Abstract The NCI series of cell lines represent a unique collection of permanent human tumor cell lines established by one laboratory over a period of approximately 16 years. More than 300 cell lines were established, mainly from human lung cancers (both small cell and non-small cell types). In addition, smaller numbers of lines were established from rare and unusual tumors such as cutaneous T cell lymphomas, myelomas and adrenal cortical carcinoma. The T cell lines played a pivotal role in the isolation of human retroviruses including HTLV-1 and HIV. The establishment of such a large panel of lines was aided by the development of defined media for culturing specific cell types. The lines are well characterized, and full clinical data are available for most of them. Many of the lines have been deposited with the American Type Culture Collection, Rockville, MD, where they are readily available for a modest handling fee. The lines have been widely distributed to investigators, and have had a major impact on biomedical research. © 1996 Wiley-Liss, Inc.

Key words: cell culture, lung carcinoma, human, retroviruses, HIV

The NCI series of cell lines represent a unique collection of permanent cultures established by one laboratory over a period of approximately 16 years. While most of the emphasis has been on the establishment and characterization of lung cancer cultures, smaller numbers of important cell lines have been established from other tumor types. The cell lines have had a major impact on biomedical research, far disproportionate to their numbers.

Why did one collection of cell lines have such a major impact, and why are they so widely used? The reasons include: (1) A large comprehensive collection of cell lines focusing on one cancer type (lung carcinoma), but representing all of the major types and many of the minor subtypes; (2) Because many of the lines were established from patients entered onto clinical protocols, complete details about their clinical course are available; (3) Establishment of other cell lines from a small group of highly interesting tumors; (4) Careful characterization of many of the lines prior to publication and distribution; (5) Retention of differentiated features by many of the lines; and (6) Ready availability of representative cell lines and their widespread distribution to interested investigators worldwide, either directly from our laboratory or from the American Type Culture Collection (ATCC), Rockville, MD. The numerous publications resulting from the widespread use of the cell lines provided further publicity and enhanced their utilization. More recently, the establishment of tumor cell B-lymphoblastoid cell lines has enhanced the usefulness of the former by providing a self-replicating source of constitutional DNA.

The roots of the collection stretch back to 1975, when one of us (J.D.M.) was appointed as head of the NCI-VA Medical Oncology Branch, part of the Clinical Oncology Program of the NCI. As it specialized in lung cancer, and because lung cancer is relatively common in veterans, the Branch was located at the Veterans Administration Medical Center, Washington, DC. Shortly thereafter he recruited A.F.G. as a section head to establish a laboratory research program in cancer biology. It had always been the strong belief of J.D.M. that laboratory investigations in such a setting should strongly support the clinical studies, so as to lead to newer diagnostic and therapeutic approaches. Small cell lung cancer (SCLC) is much more responsive to cytotoxic therapy than other forms of lung cancer (collectively known as non-small cell lung cancer or NSCLC). Thus, all of the lung

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cancer protocols at the NCI-VA Branch focused on SCLC. A.F.G. was assigned the task of establishing model systems for the study of SCLC (see below). Another major clinical interest, initiated at the NCI-VA Branch by Paul Bunn, was a study of the treatment and biology of cutaneous T-cell lymphomas. As no model systems for this disease existed, they also had to be developed. While concentrating on these two diseases, our interest extended to other endocrine secreting and certain rare tumors that we encountered during the course of our clinical studies. Some years later, at Dr. Paul Sugarbaker's urging, we established several colorectal carcinoma cultures (see below). In 1981, the Branch relocated to the Naval Medical Center, Bethesda, MD, directly across Rockville Pike from the NIH, and was renamed the NCI-Navy Medical Oncology Branch. The laboratory was transferred about 1 year later. However, the same programs and interests were maintained, with the addition of major clinical protocols for NSCLC. Thus, a systematic approach to the culture of NSCLC replaced the previous sporadic approach. In addition, the development of clinical protocols for SCLC based on in vitro drug sensitivity testing were developed. This had the effect of accessioning many clinical specimens and establishing several cell lines from previously untreated SCLC patients.

Specimens were accessioned at the Branch starting in July 1976. The first cell line, NCI-H23, was initiated from a lung adenocarcinoma resection specimen received in August 1976, from the twenty-third sample received. During the next 15 years 2,595 specimens were received, from which 325 cell lines were initiated. The success rate of 12.5% is misleading. Many of the samples were from staging procedures, such as blind bone marrow aspirates, and up to 25% of the specimens lacked tumor cells. Occasionally, two cell lines (a tumor line and a B-lymphoblastoid line) were initiated from a single specimen. Most of the cell culture work and characterization was performed in the laboratory of one of us (A.F.G.), ably assisted by Herbert Oie and Edward Russell, and initially, by Harold Stull. During the time period 1980–1983, some of the cell lines were initiated in the laboratory of Desmond Carney, assisted by Virginia Bertness, Gerold Bepler, Martin Brower, and Fabian Calvo. Persons playing crucial roles in specimen acquisition and collection of patient data included Daniel Ihde, Paul Bunn, Bruce Johnson, James Mulshine, Ilona Linnoila, Harvey Pass, Paul Sugarbaker, and Ruby Phelps. The many investigators who helped us characterize the lines are mentioned in the references. Some of them are authors of other reports in this Supplement.

The nomenclature system for the cell lines needs explanation. All specimens reaching the cell culture laboratory received a sequential number. Initially, specimens directly from patients were labeled HUT (for human tumor) and NUT (for nude mouse tumor) if they had been passaged initially as xenografts in atyhymic nude mice. Prior to the first publication of our lung cancer lines, the nomenclature system was changed. All specimens received the prefix NCI- (although several publications have failed to use this prefix). Specimens directly from patients also receive the secondary prefix H followed by a sequential number (e.g., NCI-H69). Specimens from nude mouse xenografts receive the secondary prefix N (e.g., NCI-N417). However, prior to the change in nomenclature, two important CTCL lines, HUT 78 and HUT 102, were described in the literature [1], and deposited at the ATCC (see below). Thus, the original nomenclature is still retained for these two lines, while the newer NCI nomenclature has been applied to all other human cell lines.

As previously mentioned, early and widespread distribution of the cell lines occurred. During the period 1979–1992, cell lines (usually multiple in number) were distributed by us to investigators worldwide on 877 occasions. During recent years, costs for labor, materials, and shipping charges exceeded $15,000 per year. Representative examples of the cultures were deposited in the ATCC, and thus were available to any investigator for a modest handling fee. During 1992 alone, nearly 1,000 samples of NCI cell lines were distributed by the ATCC (R. Hay, personal communication).

Lung Cancer Cell Lines

During the fifteen year period 1976–1991, about 239 lung cancer cell lines (including mesotheliomas) were initiated. They included 122 SCLC lines, and 117 NSCLC lines. As previously mentioned, initial efforts (clinical and laboratory) were focused on SCLC, with some NSCLC lines established as controls. When clinical protocols for NSCLC were developed in the early to mid eighties, systematic studies on NSCLC commenced. These lines have been extensively used for numerous studies encompassing virtually all aspects of the biology and molecular genetics of lung cancer, several of which are summarized in

the accompanying reports published in this Supplement.

While the NCI group of lines are the largest, best characterized, and most widely distributed and utilized lung cancer cell lines, groups of investigators at other institutions have established panels of cell lines. For those interested in the major contributions of others, a comprehensive review and listing of the lung cancer cell lines has recently been published [2].

Recently we have concentrated on establishing paired tumor cell-B lymphoblastoid cell lines. Over 30 such pairs currently exist, of both SCLC and NSCLC origin. "DNA fingerprinting" is used to confirm the common parentage of paired lines. These pairs provide a unique and highly useful resource, especially for investigating mutational phenomena such as allelotyping using loss of heterozygosity studies.

Because of major differences in the pathology, biology, clinical features, and culture conditions between SCLC and NSCLC, these two major subdivisions of lung cancers are discussed separately.

SCLC Lines

We started culturing SCLC in 1977, and reported the establishment of multiple cell lines in 1980 [2]. Prior to the latter date, a few individual cell lines had been described [3–5]. In addition, the Dartmouth group was simultaneously establishing multiple cell lines [6]. However, because of limited distribution, the Dartmouth lines have not been utilized as extensively as the NCI lines.

TABLE I. The NCI Series of Cell Lines

Cell type	Number
Lung cancer	223
Non-small cell	110
Small cell	108
Extra-pulmonary small cell	5
Mesothelioma	17
Gastrointestinal	16
Colorectal carcinoma	15
Gastric carcinoma	1
B-lymphoblastoid	47
Miscellaneous	7
Cutaneous T-cell lymphoma	2
Breast carcinoma	1
Esthesioneuroblastoma	1
Multiple myeloma	2
Adrenocortical carcinoma	1
Total	310

Because SCLC is seldom treated by curative intent surgery, almost all specimens we received were from metastatic sites. Most specimens were biopsies or aspirates obtained during routine diagnostic or staging procedures, and many lacked detectable tumor cells. Even in positive samples, the number of identified living tumor cells usually was low. In fact, some tumor lines were initiated from cytologically negative specimens. On some occasions, resections of lymph nodes, or intrathoracic surgical procedures yielded gram quantities of tumor tissues. However, the success rate of initiating cell lines from these larger samples was only marginally higher. Most cell lines were initiated from marrow aspirates, malignant effusions, and node resections. However, almost all sites from which we received samples were successfully cultured, except for fiberoptic bronchial biopsies, CSF, and urine.

Our initial attempts to culture SCLC (using RPMI-1640 medium supplemented with fetal bovine serum, R10) were unsuccessful. A perusal of published literature, and our own experience, indicated that tumors serially passaged in animals were easier to culture than the original tumors. We successfully cultured two nude mouse xenografts which we had received from Dr. Yukio Shimosato, National Cancer Center, Tokyo. Studying these proved useful in modifying our techniques so as to culture original tumors without animal passage. Modifications included the realization that, under our culture conditions, most SCLC cultures grow as floating cellular aggregates, that they must be maintained and passaged at relatively high density, and that stromal cells release factors that aid initial tumor growth. With these technical improvements we could establish SCLC cell lines, although most of the original lines were from patients who had relapsed after responding to initial therapy. These findings suggested that clinical relapse was associated with molecular changes in tumor cells that imparted an enhanced ability for their in vitro growth.

Establishment of some continuous cell lines directly led to major improvements in culture technique and success rate. Gordon Sato and co-workers had established that cells could be cultured for indefinite periods in defined media, and that the specific growth requirements for specific cell types varied [7,8]. However, it appeared that almost all cells required insulin-like growth factors, transferrin-like growth factors, a steroid hormone, and trace elements. Utilizing SCLC cultures established in routine serum-

supplemented media, Elizabeth Simms defined the growth requirements of SCLC cells. Within a short time she developed a serum-free defined medium that supported the continuous replication of established SCLC cultures. She identified five supplemental growth factors, selenium, hydrocortisone, insulin, transferrin, and estradiol [9]. Our original acronym for these factors, SHITE, was changed to HITES at the request of a sensitive editor. Desmond Carney utilized HITES-supplemented medium to establish cell lines directly from tumor samples [10]. While HITES medium was superior to routine media for the establishment of cultures, addition of small amounts of serum to HITES-supplemented medium sometimes resulted in increased growth advantage [11]. Because of this observation, Herbert Oie added four additional growth factors and devised 9N medium [12]. However, once vigorous in vitro of SCLC cells commenced, the growth requirements simplified. In fact, the three most important growth factors were selenium, insulin, and transferrin, with the other factors being of occasional or marginal value. These results suggested that SCLC cells frequently released peptides required for their own growth (autocrine secretion). In fact, established cell lines could be maintained in totally unsupplemented growth media indefinitely [13,14]. Culture techniques evolved until about 35% of all tumor bearing SCLC specimens could be cultured, even though many specimens contained minute numbers of tumor cells [11]. Because of these results, we developed protocols for treating SCLC patients with in vitro selected therapy [11]. In most cases, the numbers of tumor cells had to be amplified by culture prior to testing.

An unexpected benefit from the culture of SCLC specimens was the recognition of the variant form of SCLC (SCLC-v). Earlier, clinical and pathological observations had indicated that some cases of SCLC presented with mixed small cell-large cell morphology, and that these tumors had a poor response to therapy and shortened survival [15]. Cell lines started from these cases demonstrated large cell morphology, increased growth and cloning efficiency, and radioresistance [16–18]. Many of these variant lines had considerable amplification and over-expression of the c-*myc* proto-oncogene [16,19,20]. Variant morphology and c-*myc* amplification were more frequent in patients who had received prior therapy [20]. In fact, those features may be associated with specific forms of cytotoxic therapy [20]. Since switching to etoposide/cisplatin for initial therapy, the incidence of variant tumors has decreased considerably.

Tumors closely resembling SCLC may arise at a large number of organ sites [21,22]. We have established a number of cell lines from small cell carcinomas arising at a variety of extra-pulmonary sites [23–25]. These cell lines retained the NE cell phenotype characteristic of SCLC, and were highly chemosensitive. However, molecular studies confirmed that they represent a subgroup distinct from pulmonary SCLC [24, 41].

Non-Small Cell Lung Cancer (NSCLC) Cell Lines

While the first of the NCI lines was an adenocarcinoma of the lung, we did not systematically study the culturing of NSCLC until the development of appropriate clinical trials at our Branch in the early to mid eighties. While several lines had been described in the literature, including the widely used A549 cells [26], most lines were relatively or completely undifferentiated, and were not representative of the tumors from which they were derived. The culturing of NSCLC presented a different set of problems than SCLC (Table II). Most NSCLC samples were from primary tumors that had been surgically resected, and usually contained large amounts of tumor tissue. However, the diversity of NSCLC tumor cell types complicated their culture. Different forms of differentiation required different media and culture conditions. Because the precise pathological tumor type seldom was identified correctly when the specimen reached the laboratory, several media and culture conditions were utilized for the culture of a single tumor specimen, provided the sample was

TABLE II. Comparison of the Culture Features of Lung Cancers

Feature	SCLC	NSCLC
Specimen site	Usually node or distant metastasis	Usually primary tumor
Tumor cells in specimen	Usually few	Usually abundant
Number of tumor phenotypes	Relatively few (2–3)	Relatively many
Culture conditions and media	Relatively uniform	Relatively variable
Culture success rate	Relatively high	Relatively low
Prognostic effect of successful culture	None	Negative (for primary tumors)

of sufficiently large size (see article by H. Oie in this issue).

It was realized relatively early that it was easier to establish permanent cultures from adenocarcinomas and large cell undifferentiated carcinomas than from squamous cell carcinomas. Martin Brower, working with Desmond Carney, developed a serum free medium for the culture of NSCLC, especially adenocarcinomas, which was termed ACL-3 [27]. Herbert Oie modified this formula so as to improve the culture success rate and reduce its price. The resultant ACL-4 medium was found to support the growth of many different types of adenocarcinomas and certain other tumor types arising in several organ systems [28–30].

There are at least four recognized subtypes of adenocarcinoma of the lung. While some of these tumors form glands and secrete mucin, many of these arise from progenitor cells (Clara cells and Type II pneumocytes) of the peripheral airways (bronchioles and alveoli). These tumors may express markers of peripheral airway differentiation, including surfactant proteins and a Clara cell specific 10 kD protein. The tumors often have papillary features, as opposed to gland formation. In non-adherent cultures, papillary structures may resemble glands, but the apical surfaces of the cells are oriented outwards. We established several cell lines from peripheral adenocarcinomas. Several of these had papillary growth patterns, and expressed peripheral airway cell markers and characteristic intracellular structures [31–33]. These were the first human tumor lines to conclusively express these markers, even though it had been claimed that the well-known A549 cells elaborated surfactant [26].

The major problem with culturing squamous cell tumors was the fact that the fully differentiated epidermal cell is non-replicating. In fact, it is a water impermeable metabolically inert "dead" cell surrounded by a tough water impermeable shell (the cornified or cross-linked envelope) just below the outer membrane. Envelope formation requires several proteins and enzymes, and a high calcium concentration. Thus, full differentiation and cell division are incompatible. The difficulty was in finding the right balance between differentiation and division. Fast replicating lines expressed few or none of the characteristic properties of squamous cells, while well-differentiated primary cultures could not be passaged. Because organized terminal differentiation occurred in multilayered cells, one technique to prevent it was to passage cultures prior to confluence. The use of semi-defined media with reduced calcium concentration and various growth factors [28,34] resulted in the establishment of a modest number of cell lines expressing several markers of squamous differentiation, including involucrin, transglutaminase, high molecular weight keratins, up regulation of EGF receptor expression, and induction of cornified envelopes. Of interest, the lines with the most differentiated squamous markers were derived from adenosquamous tumors, and retained the capacity for dual differentiation.

Large cell carcinomas are classified as such because they lack all morphological features of differentiation, and most cell lines derived from them also lacked differentiated features. However, a few of the cultures expressed markers of other forms of differentiation, in particular gland formation and mucin secretion. Presumably these tumors represented extreme examples of poorly differentiated adenocarcinomas.

Approximately 15% of NSCLC tumors express most or all of the NE program of differentiation [23,35]. There is some evidence that patients whose tumors express NE markers respond better and live longer than other patients with NSCLC [36–38]. We have initiated several NSCLC cell lines with NE markers, especially from adenocarcinomas and large cell carcinomas. Surprisingly, these lines were highly sensitive in vitro to several cytotoxic drugs commonly used for the treatment of lung cancer [23].

A modest number of tumors have been initiated from patients with rare types of lung cancers (Table III), including carcinoids and a mucoepidermoid carcinoma [35,39]. Carcinoid tumors of either gastrointestinal or respiratory tract origin are exceedingly difficult to culture. We have initiated several cell lines from carcinoid-like tumors. However, many of these probably represent a newly described entity, large cell neuroendocrine carcinoma [40], a tumor that has morphological and clinical features intermediate between atypical carcinoids and SCLC. However, at least one line, NCI-H727, was derived from an atypical carcinoid. Molecular, cytogenetic, and in vitro drug testing studies indicate that our "carcinoid" lines represent a distinct subgroup different from SCLC and NSCLC [23,25,41].

As previously mentioned, cell lines were much easier to initiate from metastatic NSCLC tumors compared to primary lung cancers. The ability to establish in vitro tumor cell lines from primary tumors was an independent prognostic

TABLE III. NCI Cell Lines Representing Rare or Unique Tumor Cultures

Lung carcinomas	Variant form of SCLC
	Bronchial carcinoids and large cell neuroendocrine carcinomas
	Mucoepidermoid carcinoma
	NSCLC tumors with NE markers
	Extra pulmonary small cell carcinomas
	Adenocarcinomas expressing markers of peripheral airway cells
Gastrointestinal carcinomas	Well-differentiated gastric carcinoma
	Mucinous colorectal carcinoma
	Colorectal carcinoma with NE markers
Miscellaneous tumors	Cutaneous T-cell lymphoma cell lines
	IgA secreting myeloma cell line with a rearranged c-*myc* gene
	Adrenocortical carcinoma secreting multiple steroid hormones
	Esthesioneuroblastoma
	Insulin and somatostatin secreting islet cell tumor of rat origin

factor for survival in non-small-cell lung cancer [42]. By contrast, there was a lack of relationship between in vitro tumor cell growth and prognosis in extensive stage small cell lung cancer [43].

Cutaneous T Cell Lymphoma (CTCL) Lines

While only two permanent CTCL lines are included in the NCI series, their impact on medical research has been enormous. Of interest, when the first report describing their establishment and characterization was submitted to an august English journal, it was rejected on the grounds that "if it is possible to propagate long term cultures of normal helper T-cells, there is no purpose in propagating their malignant counterparts"!

One of the major clinical protocols established at the NCI-Navy Branch by Paul Bunn was for the therapy of cutaneous T-cell lymphomas. Because understanding the biology of these relatively rare tumors was an important goal of these studies, it was important to establish cell lines. At that time only a handful of T-cell lines existed, mainly from acute lymphoblastic leukemias. Our initial attempts to grow CTCL lines were unsuccessful, and it became obvious that specific growth factors or mitogens were essential. In 1978, one of us (A.F.G.) approached Frank Ruscetti in Robert Gallo's lab for assistance with the establishment of CTCL lines. Ruscetti had been a co-author on an important recent manuscript from Gallo's team, describing the isolation and properties of T-cell growth factor (later known as IL-2) [44]. IL-2 helped the long-term growth of normal helper T-cells, although the cultures remained factor dependent. Ruscetti provided seed stocks of the factor. He mentioned that with the assistance of Bernard Poiesz he was attempting to isolate retroviruses from human leukemias, and requested that they be permitted to search for viruses in any CTCL lines that we established. After some trial and error, we determined that IL-2 and several other lymphocyte mitogens could stimulate the growth of CTCL cells for lengthy periods of time [1,45]. While most of these did not become permanent cultures, two permanent lines were established [1]. These lines were HUT 78 and HUT 102, and they had the same T4 helper cell phenotype characteristic of CTCL cells. After establishment, they could be weaned from their growth factor requirements, and were subsequently maintained in routine serum supplemented medium. Others who played key roles in the establishment and characterization of these lines included Paul Bunn, Edward Russell, and Desmond Carney.

In 1979 these lines were transmitted to Ruscetti and Poiesz. Within a short time they had isolated and characterized a retrovirus from HUT 102, a cell line established from a lymph node of a patient then believed to have mycosis fungoides, using T-cell growth factor as the initial mitogen. This virus, the first bona fide human retrovirus, was named HTLV-1 (for human T-cell lymphotropic virus type 1). The association of HTLV-1 with adult T-cell leukemia was rapidly established [46]. Later, its association with an unusual form of chronic progressive demyelinating myelopathy (HTLV-1 myelopathy or tropical spastic paraparesis) was discovered [46]. In retrospect, the patient from whom HUT 102 was established suffered from adult T-cell leukemia (a condition that had not been described in 1978), instead of mycosis fungoides as originally believed.

The other cell line, HUT 78, was established from the peripheral blood mononuclear cells of a patient with Sezary's syndrome, using concanavalin A as the initial mitogen. It did not produce HTLV-1, nor did it have integrated viral sequences. These properties, along with its mature helper T-cell phenotype, made it a suitable candidate cell line for the propagation of the AIDS virus. While the French group headed by

Luc Montagnier had isolated a retrovirus from an AIDS patient, their inability to continually propagate the virus hindered research and the development of a serum test. Mikulas Popovic was assigned the task of culturing the AIDS virus by Gallo in 1982, and, after consulting with Dean Mann, selected HUT 78 for the isolation and propagation studies. Unfortunately, there were several freeze downs of HUT 78 in Gallo's lab, and HLA and lymphocyte antigen typing by Mann indicated that they represented more than one phenotype, presumably as a result of cell contamination. Popovic successfully propagated a virus isolate in HUT 78 [47]. While it was originally claimed that it represented an independent isolate, subsequent molecular studies have confirmed that it is a strain of the original French isolate sent to Gallo's lab by Montagnier. Apparently, because of uncertainty regarding the origin of the cells used for the initial isolation, Gallo's lab changed the designation of the HUT 78 subline Popovic was using to HT. Popovic then isolated a highly permissive clone, H9.

While HUT 78 cells played a pivotal role in the first continuous passage of the AIDS agent and for providing reagents used in the resultant blood test, this information was unavailable to the scientific community for some years. The original papers from Gallo's lab failed to correctly identify the origin of HT or H9 cells, or even to discuss any possible relationship to HUT 78 [47]. However, a few months later Jay Levy published a report on the use of HUT 78 cells for multiple virus isolations from AIDS patients [48]. John Crewdson, an investigative reporter for the *Chicago Tribune* was of considerable assistance in identifying the link between the cells [49], and a subsequent investigation confirmed the derivation of H9 cells from HUT 78 [50]. This episode has been described in a lengthy, detailed news feature in the journal *Science* by another investigative reporter, Ellis Rubinstein [51].

Thus, the two permanent T-cell lines established by our group played, and continue to play, an important role in the evolving history of human retroviruses.

Gastrointestinal (GI) Cell Lines

Our interest in GI tumors began when Paul Sugarbaker, who was then an NCI surgeon, began supplying primary and metastatic colorectal carcinomas. Herbert Oie established several cell lines, with an efficiency of about 45%, and determined that ACL-4 medium was more efficient than R10 for initial culture. The cell lines remained uncharacterized and neglected, until the arrival of Jae-Gahb Park, a Korean surgeon with an interest in GI tumors. Park had established some colorectal carcinoma lines in Korea, mainly from nude mouse xenografts. He characterized both sets of lines [30]. While other groups have established multiple colorectal cell lines, our lines had some distinctive features. The collection ranged from highly to poorly differentiated. Of particular interest, NCI-H716 a c-*myc* amplified line expressed the entire range of neuroendocrine cell features. Another line, NCI-H498 was from mucinous carcinoma. Under growth conditions that prevent substrate adhesion, these cells grow as floating aggregates surrounded by a halo of extra cellular mucin. Another interesting feature was cytogenetic evidence of gene amplification in many of the lines. However, efforts by several laboratories to identify the genes in the amplicons have been unsuccessful to date. The lines are heterogeneous for expression of growth factor and CEA genes [52], and proto-oncogenes, and express functional receptors for several gastrointestinal peptides [53].

Park also established several gastric carcinoma cell lines from Korean patients. He characterized these lines, along with a well-differentiated gastric line, NCI-N87, established several years previously at the NCI-Navy Branch [54]. Unlike colorectal carcinomas, gastric carcinomas are very difficult to culture, and most of the reported lines were established by Japanese researchers. Park utilized the GI lines to demonstrate that they were relevant models to study drug resistance [55–57].

Miscellaneous Other Lines

An adrenal carcinoma cell line, NCI-H295, was established from a primary tumor [58]. Adrenal carcinomas are exceedingly rare, and virtually no functioning human lines are available. The cultured cells had ultrastructural features of steroid-secreting cells and contained complex cytogenetic abnormalities including the presence of multiple marker chromosomes. Steroid analyses (radioimmunoassays and mass spectrometry), performed 7 to 9 years after culture initiation, demonstrated secretion of more than 30 steroids characteristic of adrenocortical cells. The major pathway of pregnenolone metabolism in NCI-H295 cells is androgen synthesis, with formation of dehydroepiandrosterone, androstenedione, testosterone, and at least three sulfated androgens, as well as estrogens. In addi-

tion, formation of cortisol, corticosterone, aldosterone, and 11 beta-hydroxyandrostenidione indicated the presence of 11 beta-hydroxylase. Thus, multiple pathways of steroidogenesis are expressed by NCI-H295 cells, including formation of corticosteroids, mineralocorticoids, androgens, and estrogens. These findings indicate the presence in NCI-H295 cells of all of the major adrenocortical enzyme systems, including 11 beta-hydroxylase, desmolase, 21 alpha-hydroxylase, 17 alpha-hydroxylase, 18-hydroxylase, lyase, sulfokinase, and aromatase. The NCI-H295 cell line provides a unique model system for studying the regulation, metabolic pathways, and enzymes involved in steroid formation and secretion [59,60]. In addition, it may provide insights into the biology and treatment of adrenocortical carcinoma [61].

Two myeloma cell lines were established, one of which, NCI-H929, has been described in the literature [62]. In contrast to mouse myeloma, only a limited number of lines have been established from human myelomas. Both of the NCI lines are of interest. NCI-H929 has a highly differentiated, functional phenotype, and overexpresses c-*myc* RNA [63]. A complex translocation has interrupted the third exon of the c-myc gene in NCI-H929 cells. As a result of this rearrangement, a chimeric mRNA is expressed which commences 5′ of the c-*myc* coding region and includes sequences introduced by the translocation event. This chimeric c-*myc* mRNA, in which most of the germ line c-*myc* 3′ untranslated region has been replaced, was greater than sevenfold more stable than c-*myc* transcripts with intact 3′ ends. The other cell line was derived from a patient with immunoglobulin secreting chronic lymphatic leukemia (CLL), and the cell phenotype is intermediate between mature plasma cells and CLL cells (A.F.G., unpublished data).

Another interesting, possibly unique cell line, NCI-H1011, was established from a metastatic lesion from an esthesioneuroblastoma [64]. The latter is an exceedingly rare malignant neuroectodermal tumor of olfactory epithelium origin. Cytogenetic studies indicated that the cells contained a reciprocal translocation, t(11;22)(q24; q12), indistinguishable from the one that has been reported in Ewing's sarcoma, Askin's tumor, and peripheral neuroepithelioma. The uniqueness of this marker suggested that these tumors may be derived from the same type of stem cell, with varying histopathologic and clinical manifestations.

The only non-human lines established by the NCI group is the RIN series. Because functioning islet cell tumors have been exceedingly difficult to culture, we attempted to culture a serially transplantable, radiation-induced, islet cell tumor that had arisen in a parabiotic rat. A cell line, RIN-m, was established which secretes insulin and somatostatin as well as the classic neuroendocrine cell markers [65]. Clonal analysis indicated that peptide secretion was heterogeneous while all clones expressed neuroendocrine markers [66]. High insulin and somatostatin producing clones were isolated [66], and have been widely used to study islet cell function and secretion.

Relevance of Cell Culture Systems and Their Usefulness for Biological Studies

Cell culture systems have been widely used for the study of the biology and molecular genetics of cancer. An appropriate question that has been repeatedly asked is "What is the relevance of these systems and how closely do they represent the tumors from which they were derived?" In part, these questions were asked because (1) many cell lines lack differentiated properties present in the original tumors, and (2) because clonal evolution or selection processes may have occurred during long-term culture. We have addressed these questions before [67], and we will readdress them, with emphasis on the NCI series.

One of our major original aims was to develop cultures that closely resembled the original tumors. Our emphasis has been to establish well-differentiated cell lines. Frequently, the culture conditions that permitted long-term replication of well-differentiated cells resulted in slow growing populations. We felt that this was an appropriate "trade off" for obtaining cultures whose degree of differentiation closely resembled the original tumors.

We have emphasized careful characterization and comparison of tumor and culture properties, and provided many examples of retention of differentiated characteristics during long-term culture [68]. Numerous other examples are provided in many of the other articles published in this Supplement and in the references that they quote. Paradoxically, NE cell properties are expressed at higher frequencies and concentrations in SCLC lines than in corresponding tumors. While these may represent a selective growth advantage of cells with a NE phenotype, we believe otherwise. Tumors always contain varying percentages of stromal and other non-

malignant cells, and frequently contain extensive areas of necrosis. In addition, the fraction of dividing cells in tumors is usually less than in their corresponding cultures. Tumor cultures mainly consist of viable, relatively rapidly dividing, pure tumor cell populations. Thus, tumor cultures often may be more representative of the tumor cell phenotype than tumor tissues!

Without question, tumors consist of several phenotypes, some of which are propagated preferentially in vitro. Examples of phenotypes having growth advantages include cells having activated oncogenes, such as *ras* mutations or *myc* over-expression, and those with loss or mutations of tumor suppressor genes, such as p53. Thus, myc amplified large cells preferentially replicate in vitro from mixed small cell-large cell mixtures in the tumor tissues, and the incidence of p53 mutations in SCLC cell lines is higher than in tumors [69]. However, in all cases examined, mutations present in cell lines were present in at least a proportion of the cells in tumor tissues [70,71]. Similarly, cytogenetic and biologic features first identified in cell lines have been confirmed using tumor derived materials. Examples include deletions of the short arm of chromosome 3p, first identified in SCLC lines [72], and now confirmed in a high percentage of lung cancers of all histological types [73]. Further evidence of the relevance of cell cultures, and the long-term retention of features in vitro is provided by chemosensitivity testing of cell lines. The patterns of chemosensitivity of lung cancer and GI cell lines remain characteristic of the original tumor types [11,23,57]. In fact, chemosensitivity testing of recent or long-established SCLC cultures is predictive of the patients' responses to initial therapy [11,74]. Thus, carefully characterized cell lines are suitable for screening for new chemotherapeutic agents [75].

To paraphrase our previous conclusions [67], tumors, whether they be of clonal or polyclonal origin, are dynamic processes, constantly undergoing alterations, both in vivo and in vitro. However, in many if not most tumors, certain properties are relatively stable. There must be selective advantages for tumor populations to maintain these properties. A careful comparison of the properties of tumors and their cell lines, and correlating these data with the clinical history of the tumor, is essential. Each model system needs to be studied to determine its relevance. From such studies we conclude that many cell lines are suitable models to study tumor biology and many important contributions would have been impossible without large comprehensive panels of cell lines representing a wide variety of tumor types. Finally, in vitro studies already (and will continue to) suggest newer, more rational approaches to tumor control.

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