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Perspectives and Commentaries

Cell Lines as an Investigational Tool for the Study of Biology of Small Cell Lung Cancer

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(A COMMENT ON: Engelholm SA, Vindelov LL, Spang-Thomsen M. *et al.* Genetic instability of cell lines derived from a single human small cell carcinoma of the lung. *Eur J Cancer Clin Oncol* 1985, **21**, 815–824.)

THE ARTICLE by Engelholm *et al.* [1] describes the genetic heterogeneity and instability of DNA content of a human small cell carcinoma of the lung (SCLC) cell line as determined by flow cytometry (FCM) and cloning. These studies readdress, indirectly, questions raised many times previously: how closely do continuous cell lines resemble the tumors they were derived from, and how useful are they for the study of tumor biology?

This commentary will address these questions, using SCLC as a model. In many human tumor systems inter- and intra-tumor heterogeneity has been documented [2]. In addition, major alterations in tumor morphology, genetic composition, biology, biochemistry, antigen and oncogene expression, growth rate and response to therapy may occur *in vivo* [3]. Similar alterations are well documented in SCLC tumors and they may occur relatively rapidly (i.e. within a few weeks or months). Some SCLC cell lines have been in continuous culture 10 times longer than the median survival time of SCLC patients. We must presume that tumor evolution is a dynamic process and that it occurs both *in vivo* and *in vitro*. Thus, certain alterations in some cell lines are inevitable. Because the selective pressures *in vivo* (host defences, therapy, tumor vascularization, metastatic abilities, etc.) are very different from those exerted *in vitro* (nutritional requirements, artificial environment, etc.) the nature and rate of *in vitro* alterations may be very different from those that occur *in vivo*. Each individual cell culture system must be studied in detail and its clinical relevance determined. SCLC is an excellent system for these studies as more than 100 SCLC cell lines have been established and studied intensively in several laboratories worldwide.

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DNA CONTENT

In collaboration with Drs. Paul Bunn and Desmond Carney, we have examined the DNA content of more than 200 lung cancers and 65 cell lines by FCM (Fig. 1). Approximately 25% of all lung cancers are near-diploid (DNA index or DI of 0.9–1.1), about 10% are polyclonal, and the remainder have varying degrees of aneuploidy. In almost all instances cell lines had the name DIs as their corresponding tumors. One SCLC tumor had four aneuploid peaks having DIs of 1.2, 1.35, 2.4 and 2.7. Only the former two grew in culture. In two other SCLC lines established from apparently

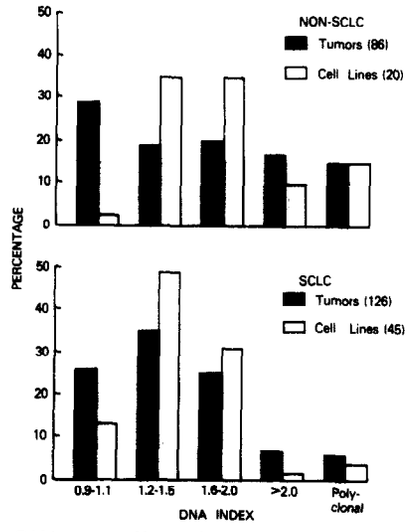

Fig. 1. DNA content of human lung tumors and cell lines, as determined by flow cytometry.

monoclonal tumors, a second aneuploid peak became apparent after long-term culture. In both instances the late appearing peak had a higher DI than the original tumor and culture. With further passage, both aneuploid populations were retained in both cultures, although the relative proportions varied. Thus, DNA contents of cell lines usually reflect those of the tumors from which they were initiated and are relatively stable.

Of interest, cell lines from non-SCLC tumors are almost always aneuploid (Fig. 1). Volm and coworkers [5] recently reported that the median survival of non-SCLC patients having aneuploid tumors was significantly shorter than those with near-diploid tumors. Thus, aneuploid non-SCLC tumors appear to have a growth advantage both *in vivo* and *in vitro*. However, the situation with SCLC tumors and cell lines is less clear.

ALTERATIONS IN MORPHOLOGY

SCLC is usually diagnosed by its characteristic morphologic features. However, some tumors at diagnosis contain non-SCLC elements, especially large cell carcinoma. Such alterations are much more frequent following therapy [3, 4]. Equivalent cell lines have been established and characterized [6, 7]. While most cell lines retain characteristic SCLC morphology and are referred to as classic lines, variant lines have varying degrees of alteration towards large cell morphology. Of 10 variant lines we have established, five were started from typical SCLC tumors, and five from combined SCLC-large cell tumors. While pathologists regarded the latter tumor group to consist of a heterogenous population, cloning studies indicate that variant lines consist of a single characteristic biochemical and biological phenotype, but have a spectrum of morphological appearances [7].

EXPRESSION OF NEUROENDOCRINE AND OTHER MARKERS

SCLC cell lines express several neuroendocrine properties including the enzymes L-dopa decarboxylase and neuron specific enlose, dense core granules and a variety of peptides, especially bombesin or its mammalian homologue gastrin-releasing peptide (BN/GRP) [6, 7]. In addition, they express high concentrations of creatine kinase, BB isoenzyme [6]. About 70% of the lines express all of these markers and they correspond to the classic subtype. The variant subtype have selective loss of some of these markers while retaining others [6, 7].

Between 70 and 95% of SCLC tumor samples express the same markers, although at 2–50-fold lower concentrations (AFG, unpublished data). There are several reasons why tumor concentrations of these markers may be lower than their corresponding cell lines: cell lines consist of relatively uniform, rapidly dividing tumor cells free of widespread necrosis, stromal contamination and sampling errors. However, the important point is that the frequency of expression of these individual markers is almost identical in tumors and cell lines.

Spatial and cloning studies indicated that the parental phenotype was retained [7, 8] regarding DNA content, morphology, neuroendocrine markers, and BN/GRP expression. However, polypeptide expression other than BN/GRP was more frequent in tumors and newly established cell lines than in long term cultures. In a study of nearly 30 cell lines, BN/GRP was expressed in all cultures over a 2-yr period providing that the initial tumor and/or early culture expressed it. In contrast, the incidence of calcitonin expression continuously decreased in long term cultures, and showed clonal heterogeneity. What is the explanation of these findings? BN/GRP is an autocrine growth factor for SCLC [9], and thus its continued secretion may be essential for *in vitro* growth. Similar findings have not been noted for other peptide products of SCLC with the possible exception of arginine vasopressin. Because BN/GRP is a neuroendocrine cell product, its expression is associated with (or requires) the other neuroendocrine properties. Thus, classic SCLC lines may have a selective pressure to retain the entire neuroendocrine machinery. Currently we are planning to test the antitumor effects of a monoclonal antibody to bombesin. Variant cell lines lack BN/GRP expression, but divide more rapidly and clone more efficiently than classic lines [7]. Variant lines may produce other growth factors, perhaps related to oncogene amplification and expression (*vide post*).

ONCOGENE AMPLIFICATION AND EXPRESSION

Variant cell lines are frequently (7/9) associated with great amplification and over-expression of the *c-myc* oncogene [7,10]. Because inappropriate regulation of *c-myc* frequently results in a selective growth advantage [11], variant lines grow more vigorously *in vitro* than classic lines (7, 10]. In contrast to the frequent finding of *c-myc* amplification in cell lines, to date we have not found amplification in any SCLC tumor sample (B. Johnson, personal communication). We presume that the tumors were heterogenous and that the variant cells with *c-myc* amplification were selectively propagated *in vitro*. These presumptions are supported by our studies for *N-myc* and other *myc* family oncogenes [11–13]. Using a human *N-myc* probe, our laboratory has identified five SCLC lines which were amplified for and expressed the *N-myc* gene. In contrast to *c-myc*, *N-myc* amplification was detected in three patient specimens. In one autopsy case, multiple metastases were examined. While all metastatic foci had *N-myc* am-

plification, there was heterogeneity of genomic size (5.5 kb and 2.0 kb bands). The cell line, established from a bone marrow aspirate during life, revealed amplification of both hands. In one other SCLC line, established from a primary, untreated tumor having *N-myc* amplification, further amplification occurred sequentially during early passage. With other cell lines, spatial and cloning studies suggest that oncogene amplification is stable after the cell line has been established. Thus, while oncogene amplification is heterogenous in tumors, selective growth of these subpopulations occurs in vitro (and perhaps in vivo). In addition, SCLC cell lines were used to recently identify the *L-myc* gene, the latest member of the *myc* family, now known to be amplified in certain SCLC tumors and cell lines.

CHEMO- AND RADIOSENSITIVITIES OF SCLC CELL LINES

Our laboratory and others have previously demonstrated that classic SCLC lines are relatively radiosensitive and have low extrapolation numbers [14], while variant lines are relatively radioresistant. Cell lines established from untreated SCLC tumors are usually sensitive to a number of cytotoxic drugs, while lines from previously treated patients are resistant to most drugs [14, 15]. All of these findings are compatible with our knowledge of the responses of treated and untreated SCLC patients and of those with variant ('combined small cell-large cell') morphology.

SUMMARY

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.

From such studies we conclude that cell lines are suitable models to study the biology of SCLC and many important contributions would have been impossible without a large comprehensive panel of cell lines. These lines may be suitable for the selection of the best in vitro regimen to treat individual patients from whom the lines were derived, a hypothesis currently being tested in our Branch. Finally, *in vitro* studies already (and will continue to) suggest newer, more rational approaches to tumor control.

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