Specific Chromosome Defect Associated with Human Small-Cell Lung Cancer: Deletion 3p(14–23)

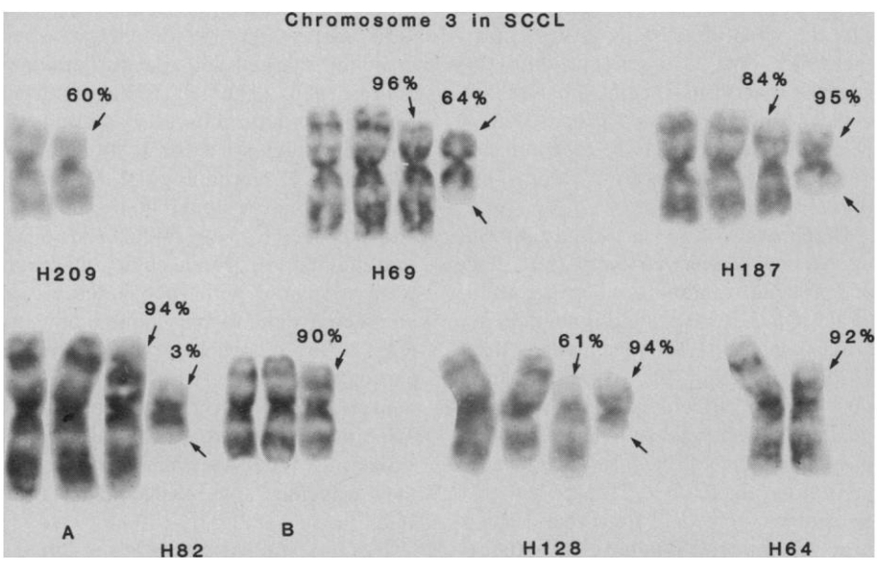
Abstract. *A specific, acquired chromosomal abnormality (deletion 3p) has been found in at least one chromosome 3 in 100 percent of the metaphases in 12 of 12 cell lines cultured from human small-cell lung cancer tissue and in 2-day tumor culture specimens from three patients. Analysis of the shortest region of overlap shows the deletion to be 3p(14–23). This specific change was not seen in five of five lung cancer cell lines other than small-cell lung cancer or in two lymphoblastoid lines cultured from cells of small-cell lung cancer patients whose tumors had the 3p deletion.*

Small-cell carcinoma of the lung (SCCL), which accounts for about 25 percent of the 110,000 new cases of lung cancer that occur yearly in the United States, is clinically distinct from the other histologic types of lung cancer (*1–3*). Usually it is metastatic at presentation, so that surgery is not used; in contrast to the other types of lung cancer, it is sensitive to chemotherapy and radiation, which are the recommended forms of primary therapy and result in cures in some cases. Prior work has demonstrated distinct biochemical markers of SCCL (*3*).

Tumor specimens were obtained during clinical and pathologic evaluation of 14 SCCL patients who had been assigned to approved therapeutic regimens at the National Cancer Institute–Veterans Administration Medical Oncology Branch. Cultures were initiated from primary tumors, metastases to bone marrow, pleural fluid, or subcutaneous tissue. Tumor cell lines, initiated and maintained in RPMI-1640 medium supplemented with 10 percent fetal bovine serum without antibiotics (*1*), had been in culture for 4 to 96 months. Five lines were started from previously untreated patients. For short-term culture, three fresh specimens were obtained and prepared as for the establishment of cell lines and were cultured for 2 days in serum-free medium supplemented with hydrocortisone, insulin, transferrin, estradiol, and selenium (HITES) (*2*). This chemically defined medium has allowed the selective growth of SCCL but not of normal cells, and the mitotic activity of the tumor cells in this type of culture is relatively high (*2*). One of these samples was also studied after 8 months in culture (as line NCI-H220). Five lines that had been established from lung cancer cells other than the small-cell type (two adenocarcinomas, two mesotheliomas, and one large-cell carcinoma) and that had been in culture from 10 to 48 months were also studied (*1*).

The SCCL lines are characterized by a continuous growth in tissue culture, ability to clone in soft agarose, formation of heterotransplanted tumors in nude mice with histology similar to that of the patients' tumors, and expression of human isoenzymes (*1*). All of the SCCL lines grow as aggregates of floating cells and show SCCL cytology with light microscopy. The high specific activities of L-dopa decarboxylase, creatine kinase-BB, and neuron-specific enolase and the dense core granules seen with electron microscopy are characteristic of SCCL (*1, 3*). The fresh tumor specimens showed SCCL on histologic examination, and the cytology of the tumor cells in the 2-day cultures was typical of SCCL.

Chromosome studies were carried out with the air-dried technique. The slides were then stained with conventional Giemsa, Giemsa-trypsin, and C-banding stains (*4*). The chromosome distribution in these 12 SCCL lines showed five to be hypodiploid, with one having near tetraploid cells as well; three lines were hyperdiploid, three were tetraploid, and one had chromosome numbers between tetraploid and octaploid. Two distinct stem lines were present in two instances. The most frequent structural abnormalities were in chromosomes 3, 1, 2, and 10. In all of the SCCL lines, 100 percent of the metaphases had a 3p deletion in at least one chromosome 3. The percentage of number 3 chromosomes involved and the structural abnormalities found in six of the SCCL lines is shown in Fig. 1. Determination of the shortest region of overlap in all of the cell lines shows this deletion to be the region 3p(14–23) (Fig. 2). The fresh tumor specimens cultured for 2 days in HITES had the 3p deletion in 100 percent of metaphases, but the abnormality was not found in two B lymphoblastoid lines (NCI-H128BL and NCI-H209BL) autologous with two SCCL lines (NCI-H128 and NCI-H209) that had the 3p deletion. The five non-SCCL lung cancer lines also did not have a deletion involving this 3p region. Therefore, we propose that the deletion 3p(14–23) is a specific, acquired, chromosomal abnormality for SCCL, existing in the tumor cell but not in other cells, such as those of lymphoid origin. Whether the chromosomal defect is present in bronchial epithelial cells, the precursor cells of SCCL (presumed to be the Kultschitzky cell), or preneoplastic lesions remains to be determined.



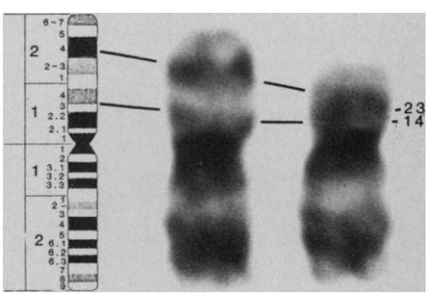


Fig. 1 (left). A partial karyotype showing structural abnormalities of chromosome 3 for six SCCL lines. The percentage of each marker is shown. Fig. 2 (above). (Right) Chromosome 3 from line NCI-H64 is shown with deletion 3p(14–23). (Left) A normal chromosome 3 with the idiogram.

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Wurster-Hill and Maurer (*5*) reported cytogenetic studies of metastatic cells in the bone marrow of 26 patients with SCCL. No mention of chromosome 3 involvement is made in 18 patients with karyotypic abnormalities, but in two of five karyotypes shown in their report, the deletion of 3p is easily seen. Any discrepancies in their findings and ours need to be resolved by further studies of SCCL by several investigators. However, our findings of the 3p deletion in SCCL from 14 different patients, including tumor specimens freshly obtained after 2 days of culture, provide strong evidence for the association of the 3p(14–23) deletion with SCCL. In addition, one of our lines (NCI-N230) was derived from a Japanese SCCL tumor heterotransplanted into a nude mouse and then given to us by Shimosato (*6*). Thus, at least one example of the 3p(14–23) deletion has been found in an Asian SCCL. Chromosome studies of other neoplasms have not shown specific abnormalities of chromosome 3. The presence of this specific abnormality may prove to be a valuable aid in diagnosis, selection of therapy, and prognosis of lung cancer, with cytogenetic as well as light microscopic criteria used for the typing of lung cancer. When more is learned about the genes present in the 3p(14–23) region, their role in the genesis and maintenance of SCCL can also be determined.

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References and Notes

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Is the Acetylcholine Receptor a Rabies Virus Receptor?

Abstract. *Rabies virus was found on mouse diaphragms and on cultured chick myotubes in a distribution coinciding with that of the acetylcholine receptor. Treatment of the myotubes with α-bungarotoxin and* d*-tubocurarine before the addition of the virus reduced the number of myotubes that became infected with rabies virus. These findings together suggest that acetylcholine receptors may serve as receptors for rabies virus. The binding of virus to acetylcholine receptors, which are present in high density at the neuromuscular junction, would provide a mechanism whereby the virus could be locally concentrated at sites in proximity to peripheral nerves facilitating subsequent uptake and transfer to the central nervous system.*

Rabies virions have been detected within axons of animals infected with rabies virus, and their transport by axoplasmic flow has been described (*1–4*). Although this indicates a role for peripheral nerves in the pathogenesis of this disease, the receptor sites for virus binding in the peripheral nervous system have not been defined. We have recently shown that, 1 hour after intramuscular injection of mice with rabies virus (strain 1820B), antigen can be detected by immunofluorescence in leg sections at cholinesterase-positive sites which probably represent neuromuscular junctions (*5*). A similar distribution of radioactively labeled rabies virus (strain CVS) was seen at 6 hours after infection (*5*). These results suggested that rabies virus is present at the neuromuscular junction shortly after inoculation of virus. In this report we confirm that rabies virus binds initially at neuromuscular junctions and that the virus appears in nerves shortly thereafter. We also present data that suggest that virus-specific host-cell receptors for rabies virus are located at, or near, acetylcholine (ACh) receptor sites on cultured myotubes from chicken embryos.

Diaphragms and attached phrenic nerves were removed from both male and female random-bred mice (CF-1 strain, Charles River) and immersed in a 5-ml suspension of rabies virus strain 1820B containing 10^6 LD_{50} (the lethal dose for 50 percent of infant mice inoculated intracerebrally). The origin and passage history of this virus strain in our laboratory have been described (*5*). Specimens were held for either 30 minutes, 45 minutes, 60 minutes, 2 hours, or 4 hours at 23°C, after which each was washed extensively in phosphate-buffered saline (PBS), *p*H 7.2, and stained with fluorescein isothiocyanate (FITC)–conjugated antibody to rabies virus (Bio Quest, Cockeysville, Maryland; FITC-Rab, diluted 1:40 in PBS). Some stained preparations were counterstained for acetylcholinesterase (*6*), which is present at high concentrations at the neuromuscular junction. Other diaphragms, not exposed to virus, were stained with rhodamine-conjugated α-bungarotoxin (R-BTX) to determine the distribution of ACh receptors (*7*).

After 30 minutes of exposure to rabies virus, the mouse tissue showed the presence of virus antigen at sites that also stained for acetylcholinesterase. Virus antigen was not distributed over the entire neuromuscular junction at this time, nor always at later time periods. However, after 60 minutes of incubation in the virus suspension, the diaphragm often exhibited marked site-specific immunofluorescence. The FITC-Rab stained some of the neuromuscular junctions on the diaphragm so extensively that the characteristic morphology of these junctions was readily apparent by immunofluorescence (Fig. 1A). When the FITC-Rab–stained neuromuscular junctions were compared with such junctions on uninfected diaphragms stained only by R-BTX, the morphologic similarity was particularly obvious (Fig. 1B). After 4 hours of exposure to rabies virus, mouse diaphragms showed FITC-Rab–stained antigen in small peripheral nerves as they traversed the muscle fibers (Fig. 1C).

We also studied rabies virus binding

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