

Involvement of Chromosome 7 in Primary Lung Tumor and Nonmalignant Normal Lung Tissue¹

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ABSTRACT

By using the newly developed adhesive tumor cell culture system, we analyzed the chromosomal constitutions of primary lung tumor and nonmalignant normal lung tissue from 10 previously untreated patients with non-small cell lung cancer. Chromosomal analyses were successfully carried out in banded chromosome preparations from 10 tumor and 8 normal lung tissue samples. All analyzed tumor and normal lung tissue samples had a predominantly normal diploid chromosome number. However, there was at least one structural or numerical alteration in every tumor and lung tissue sample analyzed. Chromosomes 1, 3, 4, 6, 7, 8, 9, 12, 15, and 20 were more often involved in rearrangement. The most consistent finding was trisomy 7; 4 patients had trisomy 7 in both tumor and normal lung tissue, and another 2 had this anomaly in tumor tissue only. Of the 4 patients without trisomy 7, 2 had a homogeneously staining region in the short arm of chromosome 7 in tumor tissue. Phytohemagglutinin-stimulated peripheral blood lymphocytes from 7 patients, including 5 patients with trisomy 7 in tumor tissue, did not show trisomy 7. These cytogenetic data suggest that chromosome 7 may be associated with lung cancer development and that trisomy 7 may be the hallmark of premalignant changes, at least in a subgroup of patients with non-small cell lung cancer.

INTRODUCTION

Nonrandom chromosomal abnormalities have been observed in many types of human hematological neoplasms and in certain solid tumors (2, 3). However, only a few cytogenetic studies on NSCLC⁴ have been reported (4-14), even though lung cancer is the most common cause of cancer deaths and 149,000 new cases of lung cancer were expected in the United States in 1986 (15). Most of these studies have dealt with metastatic tumor cells in pleural effusion (4-7, 10), and the cytogenetic changes in those tumor cells were so complex that no consistent chromosomal abnormalities have been identified.

Recently, a new monolayer culture system was developed and successfully used for the culturing of fresh human tumor cells from various tissues of origin, including lung cancer (16). We analyzed the chromosomal constitutions of primary tumor and nonmalignant normal lung tissue from the same patients after culturing them using this culture system for approximately 2 weeks. Here we report the results of successful cytogenetic analysis of 10 primary lung tumors and 8 nonmalignant normal lung tissue samples from patients with NSCLC.

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⁴ The abbreviations used are: NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor.

MATERIALS AND METHODS

Primary lung tumor and nonmalignant normal lung tissue samples were obtained from 10 previously untreated patients (5 men, 5 women) immediately after surgery (6 pneumonectomies, 4 lobectomies) according to institutional guidelines. Nonmalignant normal lung tissue samples were taken from the far margins of resection of the lung and were free of tumor grossly and microscopically.

Each tumor and lung tissue sample was cultured in a 6-well culture plate (Costar, Cambridge, MA) coated with a cell-adhesive matrix from Lifetrac, Ltd. (Irvine, CA) according to the method of Baker *et al.* (16). All tissue samples were minced with scalpels to 1-mm pieces and then enzymatically disaggregated to single cells by incubating with 0.075% collagenase type III (Cooper Biomedical, Malvern, PA) and 0.005% DNase (Sigma Chemical Co., St. Louis, MO) in Ham's F-12 (K. C. Biological, Lenexa, KS) with 10% fetal calf serum for 16 h with constant stirring. Each culture well was inoculated with 100×10^3 viable cells in 4 ml of attachment medium. After 24 h of incubation, the attachment medium was removed, the adherent cells were washed with phosphate-buffered saline, and 4 ml of culture medium were added to each well. All culture wells were refed by an 100% medium exchange after 6 and 12 days of incubation.

Culture medium was Ham's F-12 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (2.7 mg/ml) (Sigma), 10% swine serum (J. R. Scientific, Woodland, CA), and penicillin-streptomycin (100 units/ml) (GIBCO, Grand Island, NY) supplemented with the following hormones: transferrin (10 μ g/ml), hydrocortisone (0.5 μ g/ml), epidermal growth factor (5 ng/ml), and insulin (5 μ g/ml) (Collaborative Research, Lexington, MA). Attachment medium was the same formulation as the culture medium plus 0.6% methylcellulose 4000 (Fisher Scientific, Houston, TX).

Thirteen-day-old cultures were harvested for chromosomal preparations according to the procedure described elsewhere (17). If cells did not grow sufficiently to cover the surface of the well, they were left in the incubator to grow for 7 to 10 more days and harvested in the same manner 24 h after a change of culture medium. Chromosome numbers were counted on 4 to 50 metaphase spreads (median, 50), and 4 to 36 banded metaphase spreads (median, 30) were analyzed for each sample. Chromosomal abnormalities were designated according to the short version of the designation system formulated at the Paris conference (18). An abnormal clone was defined by the presence of identical anomalies in 2 or more metaphase spreads. To exclude the possibility of a constitutional chromosome abnormality, we analyzed the chromosomal constitutions of phytohemagglutinin-stimulated lymphocytes from 7 patients (cases 1, 4, and 6 to 10). Ultrastructural characteristics of the cultured cells from tumor and normal lung tissue samples were studied with electron microscopy in 2 patients (cases 9 and 10) according to a previously described procedure (19).

RESULTS

Histopathologically, 5 patients had adenocarcinoma, 4 had squamous cell carcinoma, and 1 had atypical carcinoid tumor. No one had previously received chemotherapy or radiotherapy, and all patients except cases 1, 4, and 8 had a heavy smoking history. Two normal lung tissue samples did not grow in the culture. All analyzed tumor and normal lung tissue samples had a predominantly normal diploid chromosome number, but there was at least one structural or numerical chromosomal

alteration in every tumor and normal lung tissue sample analyzed. Chromosomes 1, 3, 4, 6, 7, 8, 9, 12, 15, and 20 were more often involved in structural or numerical rearrangements.

Although many chromosomal changes were observed, the most consistent chromosomal abnormality was trisomy 7 as shown in Table 1, which was observed in approximately 10% of metaphase spreads analyzed. Four patients (cases 4, 7, 8, and 9) had trisomy 7 in both normal lung (Fig. 1) and tumor tissue (Fig. 2), and another 2 patients (cases 1 and 5) had the same abnormality in tumor tissue only. Of particular interest is that 3 of the 4 patients with squamous cell carcinoma had trisomy 7 in both normal lung and tumor tissue (cases 7, 8, and 9). In addition, a metaphase spread of the tumor sample from a patient with trisomy 7 in the normal lung tissue had an additional marker chromosome (case 4) (Fig. 2). Another tumor sample (case 8) had a metaphase spread showing trisomy 7 with structural rearrangements, $t(7;15)(q11;p11)$, suggestive of clonal evolution.

Of the 4 patients not exhibiting trisomy 7, 2 had a homogeneously staining region in the short arm of chromosome 7 (partial trisomy?) in the tumor tissue (cases 3 and 6) (Fig. 3).

Table 1 Clonal chromosomal abnormality in primary lung tumor and nonmalignant normal lung tissue

Case	Sex	Age	No. of mitoses analyzed (abnormal/total)		Clonal chromosomal abnormality	
			Tumor	Normal lung	Tumor	Normal lung
<i>Carcinoid tumor</i>						
1	F	65	4/16	1/4	47,XX,+7	—
<i>Adenocarcinoma</i>						
2	M	58	1/8	NA ^a	—	NA
3	M	70	6/15	NA	—	NA
4	F	42	8/34	4/31	47,XX,+7	47,XX,+7
5	M	60	6/32	4/29	47,XY,+7	46,XY,9q+
6	F	59	3/31	7/32	—	—
<i>Squamous cell carcinoma</i>						
7	F	67	4/12	8/32	— ^b	47,XX,+7
8	F	66	6/34	10/36	47,XX,+7	47,XX,+7
9	M	60	11/29	1/8	47,XY,+7	— ^b
10	M	58	7/31	6/30	—	—

^a NA, not analyzed.

^b One mitotic spread showed trisomy 7.

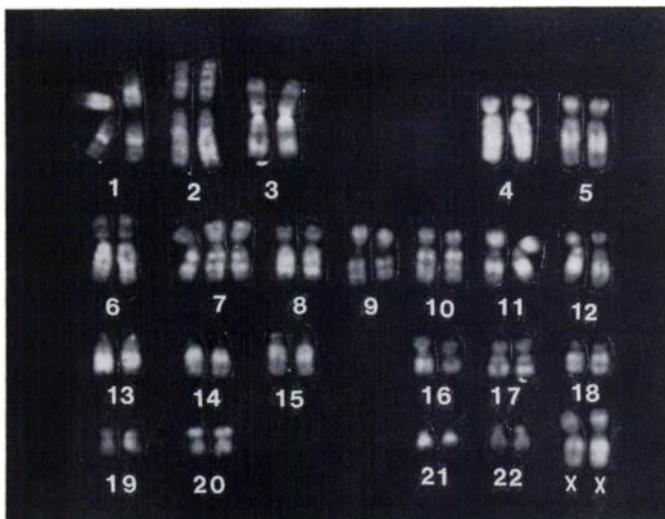


Fig. 1. Complete karyotype of a Q-banded metaphase from nonmalignant normal lung tissue (case 4) showing 47,XX,+7.



Fig. 2. Complete karyotype of a G-banded metaphase from a primary tumor from case 4 showing 48,XX,+7, and an additional unidentified marker chromosome (UM).

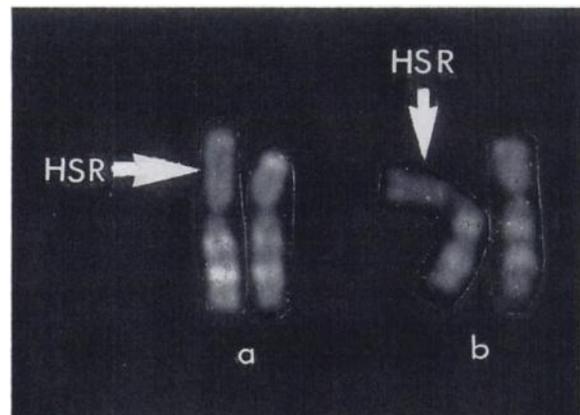


Fig. 3. Partial karyotype of Q-banded metaphases from primary tumors from case 3 (a) and case 6 (b), showing a homogeneously staining region (HSR) in the short arm of chromosome 7. A normal chromosome 7 is seen on the right side of each pair.

Two patients each (cases 3 and 7) had a metaphase spread with 4 copies of chromosome 7 and near-triploid chromosome number in their tumor tissue. Other chromosomal abnormalities included 9q+ (case 5, lung tissue), trisomies of 12 and 20 (case 6, lung tissue), trisomy 9 (case 8, tumor) and trisomy 15 (case 9, tumor). A single metaphase spread with trisomy 12 was also seen in the tumor tissue of cases 4 and 5 and in the normal lung tissue of cases 6, 7, and 8. Phytohemagglutinin-stimulated peripheral blood lymphocytes did not show trisomy 7 in any of the 7 patients studied, 5 of whom had trisomy 7 in their tumor tissue.

The electron microscopic study of the cultured tumor and normal lung tissue from case 9 showed intracytoplasmic tonofilaments (Fig. 4), indicating the squamous cell origin of the tumor. Cultured tumor and lung tissue from case 10 did not show any ultrastructural characteristics of differentiation.

DISCUSSION

In this report we have described a nonrandom chromosomal abnormality trisomy 7 in a group of patients with NSCLC and

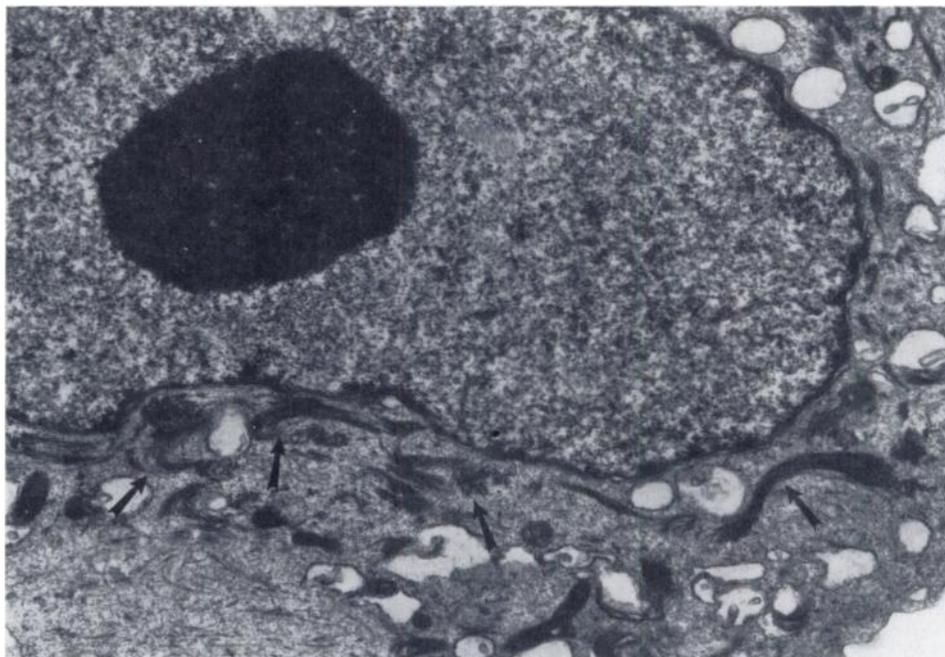


Fig. 4. Electron micrograph of cultured squamous cell carcinoma (case 9), showing bundles of tonofilaments (arrows). Uranyl acetate and lead citrate stain; original magnification, $\times 6000$.

a patient with atypical carcinoid tumor. One may certainly argue that trisomy 7 might have been a culture artifact. However, further cytogenetic studies of other primary tumors grown under the same culture conditions have demonstrated that that is an unlikely explanation for the abnormality. Among 10 primary breast tumors and 8 adjacent normal breast tissue samples studied, trisomy 7 was observed in only 2 breast tumor specimens and 1 normal breast tissue sample (20). If the trisomy 7 were induced in the culture system and if it occurred in 10% of cells as observed, there would be a better-than-95% chance of seeing one or more metaphase spreads with trisomy 7 in 29 consecutive metaphase spreads analyzed. The absence of trisomy 7 in cases 6 and 10 and in most breast tumor samples, for which more than 29 metaphase spreads of each sample were analyzed, strongly suggests that the culture system used is not the reason for this numerical chromosomal abnormality.

Another point of concern has been the potential growth of fibroblasts in the monolayer culture system. To test this possibility, 3 fibroblast cell lines were cultured under the same culture conditions. One fibroblast cell line did not adhere to the surface and failed to grow. The other 2 adhered poorly to the surface and yielded only a few cells without a single metaphase spread after 19 days of culture, suggesting that potential contamination of the tumor cell cultures by fibroblasts may not be the major problem. Furthermore, the documentation of tonofilaments within the cultured tumor cells (Fig. 4) has provided more positive evidence that the present culture system supports the growth of tumor cells.

The significance of trisomy 7 observed in both tumor and nonmalignant normal lung tissue remains to be further explored. However, one obvious consideration is that trisomy 7 in normal lung tissue may represent an early stage of lung cancer development. Teyssier *et al.* (12) described a case of carcinoid tumor with trisomy 7 as the sole chromosomal abnormality and speculated that it might be the early index of malignant transformation.

Since trisomy 7 has been observed in many other solid tumors including malignant melanoma, brain tumors, breast cancer, and colon cancer (2), it may not be an anomaly specific to lung cancer. Nevertheless, recent observation of trisomy 7 in pre-

malignant lesions such as villous adenoma of the colon (21) and colonic mucosa from individuals with familial polyposis (22) strongly supports the idea that an extra copy of chromosome 7 may play a crucial role in the early stages of tumorigenesis. A possible analogy may be drawn from experimental studies. The cytogenetic study of spontaneously transformed Chinese hamster cells *in vitro* showed that chromosome 5 trisomy preceded detectable transformation and was the first step leading to the frank malignant state (23). Association of trisomy 15 in mouse chromosomes with murine T-cell leukemias induced by various chemical or physical carcinogens or by viruses is another example (24).

If only a few strategic chromosomes are involved in the early stages of malignant transformation (12, 25), then human chromosome 7 is likely to be one of them. Located in this chromosome are several genes critical to cell proliferation and chromatin functions (26). Several cellular protooncogenes, including *erbB* (27), *met* (28), and *hA-raf-2* (29) are also located in chromosome 7. Of particular interest is the EGFR gene because the EGFR has a striking homology to the transforming protein of the *v-erbB* oncogene of the avian erythroblastosis virus (30), and several lines of evidence suggest that an EGFR-related growth regulatory system is involved in both normal and neoplastic cellular proliferation (31).

Recent studies on EGFR levels in lung cancer have shown that most squamous cell carcinomas of the lung and some other cell types of NSCLC have greater EGFR levels than normal skin, adjacent lung tissue, and small cell lung cancer (32–35). High EGFR levels have also been observed in other squamous cell carcinomas (34, 36), breast cancer (37–39), brain tumors (40–41), malignant melanoma (42), and transitional cell carcinoma of the bladder (43). There was a close correlation between increased EGFR levels and an extra copy of chromosome 7 in malignant melanoma (42).

Taken together with the multistep carcinogenesis model (44) and the observation that normal lung tissue in sections containing lung cancer does not bind EGFR antibody (33, 35), it seems reasonable to postulate that the extra copy of chromosome 7 in nonmalignant normal lung tissue may represent the early stage

of tumorigenesis requiring activation for EGFR expression by another as yet unknown factor.

Studies are in progress to correlate the cytogenetic alterations with molecular biological changes. Preliminary results have shown that the EGFR gene was overexpressed in squamous cell carcinomas of the lung without gene amplification or rearrangement (45). In addition, trisomy 7 was observed in 2 tumor samples that had high EGFR levels.⁵ If confirmed by further studies, these observations may lead to a better understanding of the development of lung cancer and potentially other solid tumors.

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⁵ Unpublished data.