

RECURRENCE OF BRONCHIOLOALVEOLAR CARCINOMA IN TRANSPLANTED LUNGS

ROBERT I. GARVER, JR., M.D., GEORGE L. ZORN, M.D., XING WU, PH.D., DAVID C. MCGIFFIN, M.D.,
K. RANDALL YOUNG, JR., M.D., AND NANETTE B. PINKARD, M.D.

ABSTRACT

Background Bronchioloalveolar carcinoma is a distinctive subtype of typical adenocarcinoma of the lung that tends to metastasize widely throughout the lungs but less commonly elsewhere. Because conventional therapies for intrapulmonary metastatic bronchioloalveolar carcinoma are generally ineffective, we treated seven patients who had intrapulmonary metastatic bronchioloalveolar carcinoma with lung transplantation.

Methods Seven patients with biopsy-proved bronchioloalveolar carcinoma and no evidence of extrapulmonary disease received transplants of either one or two cadaveric lungs. At transplantation, all native lung tissue was removed and replaced with a donor lung or lungs. The patients received the usual post-transplantation care given at the institution.

Results Four of the seven patients had recurrent bronchioloalveolar carcinoma within the donor lungs; the recurrences appeared from 10 to 48 months after transplantation. All recurrences were limited to the donor lungs. Histologic and molecular analyses showed that the recurrent tumors in three patients originated from the recipients of the transplants.

Conclusions Lung transplantation for bronchioloalveolar carcinoma is technically feasible, but recurrence of the original tumor within the donor lungs up to four years after transplantation was common. (N Engl J Med 1999;340:1071-4.)

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BRONCHIOLOALVEOLAR carcinoma is a variant of the adenocarcinoma form of lung cancer (commonly referred to as typical adenocarcinoma). The main pathological findings in this variant are cells with features of a well-differentiated adenocarcinoma, which line alveolar walls, and the preservation of interstitial structures.¹ Bronchioloalveolar carcinoma has been diagnosed with increasing frequency over the past few decades^{2,3} and now accounts for 3 to 4 percent of cases of non-small-cell lung cancer.^{4,5} According to estimates of the incidence of lung cancer,⁶ 5000 to 7000 new cases of bronchioloalveolar carcinoma will be diagnosed in the United States this year.

There is limited but convincing evidence that bronchioloalveolar carcinoma is distinct from typical adenocarcinoma of the lung. A National Cancer Institute survey of 2382 patients with bronchioloalveolar carcinoma found a substantially higher five-year survival rate among patients with all stages of the

variant than among patients with typical adenocarcinoma.⁴ The distribution of metastases in the two neoplasms also differs. As compared with typical adenocarcinoma, bronchioloalveolar carcinoma has a higher incidence of metastasis within the lung⁷ and a lower incidence of brain metastasis.⁸

We studied the outcome of treatment of seven patients with stage IV bronchioloalveolar carcinoma who underwent lung transplantation.

METHODS

Study Design

This study was approved by the institutional review board of the University of Alabama at Birmingham. Candidates for the procedure were required to have biopsy-proved bronchioloalveolar carcinoma and no evidence of extrapulmonary disease. At surgery, one or both of the patient's lungs were removed and replaced by one or both lungs from a cadaveric donor by conventional methods of lung transplantation.⁹ Post-transplantation care was identical to that of other patients undergoing lung transplantation at the institution. Recurrences were detected radiographically, with histologic confirmation in large surgical biopsy specimens, although one case (in Patient 7) was detected incidentally during a transbronchial biopsy performed for routine surveillance.

Genetic Analysis of Original and Recurrent Bronchioloalveolar Carcinoma

To determine the origin of the recurrences, amplification of polymorphic dinucleotide repeats of the genome ("microsatellite" DNA) from the original and recurrent tumors was performed in three of the patients (Fig. 1). Tissue from the tumors was obtained from rehydrated tissue sections; regions of the sections that contained only tumor were identified and specifically excised. The tissue was incubated in 50 mM TRIS-hydrochloric acid (pH, 8.3), 1 mM ethylenediaminetetraacetic acid, 0.5 percent polyoxyethyl- enesorbitan monolaurate, and 500 μ g of proteinase K per milliliter; the nucleic acid fraction was extracted by standard techniques. Microsatellite primer set DIS1728 (Research Genetics, Huntsville, Ala.) was mixed with the DNA-containing template from the slides in a mixture that contained *Taq* polymerase-chain-reaction buffer, 2 mM magnesium chloride, 1 mM deoxyadenosine triphosphate, 1 mM deoxyguanosine triphosphate 1 mM deoxythymidine triphosphate, 0.5 mM deoxycytosine triphosphate, and 0.5 μ l of [α -³²P]deoxycytosine triphosphate (1000 to 3000 Ci per millimole; Dupont/NEN, Wilmington, Del.) in a total volume of 25 μ l. The amplified fragments were size-fractionated by electrophoresis on a 4 percent Sequel gel (American Bioanalytical, Natick, Mass.) and then autoradiographed.

From the Departments of Medicine (R.I.G., X.W., K.R.Y.), Surgery (G.L.Z., D.C.M.), and Pathology (N.B.P.), University of Alabama at Birmingham; and the Lung Transplant Program (G.L.Z., D.C.M., K.R.Y.) and Lung Cancer Program (R.I.G., G.L.Z.), UAB Hospital, Birmingham, Ala. Address reprint requests to Dr. Garver at the School of Medicine, University of Alabama at Birmingham, 701 South 19th St./LHRB 339, Birmingham, AL 35294, or at robgarver@sprintmail.com.

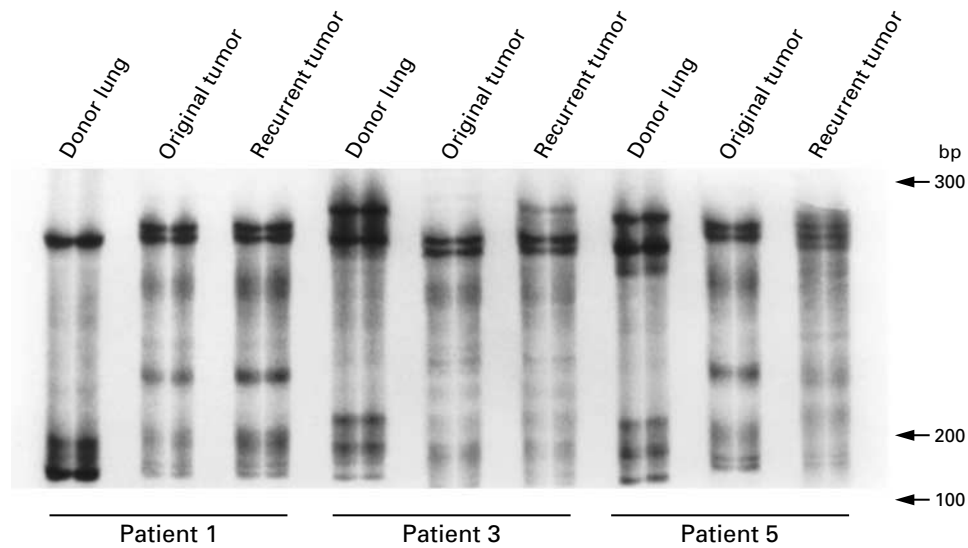


Figure 1. Genetic Characterization of Original and Recurrent Bronchioloalveolar Carcinoma.

An autoradiograph shows size-fractionated DNA fragments generated from the amplification of a microsatellite region of the genome. The source of the DNA template is shown above each lane. Lymphocyte DNA from each lung donor is paired with DNA from the original and recurrent tumors for each patient.

TABLE 1. COURSES OF PATIENTS WITH BRONCHIOALVEOLAR CARCINOMA BEFORE AND AFTER LUNG TRANSPLANTATION.

PATIENT NO.	AGE/SEX*	HISTOLOGIC† FINDINGS	RISK FACTORS	TYPE OF CONVENTIONAL THERAPY (DATE)‡	RECURRENCE AFTER CONVENTIONAL THERAPY (DATE)	TYPE OF LUNG TRANSPLANT (DATE)§	MONTHS WITH NO EVIDENCE OF DISEASE AFTER TRANSPLANTATION	RECURRENCE AFTER TRANSPLANTATION (DATE)
1	40/F	M-	None	Lobectomy (5/93)	Yes (9/93)	Double (12/93)	39	Yes (3/97)
2	33/F	M-	None	Pneumonectomy (9/92)	Yes (8/93)	Single (2/94)	56	No
3	47/M	M+	None	Double lobectomy (8/90)	Yes (1/92)	Double (4/94)	48	Yes (5/98)
4	52/M	M-	Smoked cigarettes (36 pack-years)	None	—	Double (2/95)	43	No
5	48/F	M+	None	Pneumonectomy (11/94); external-beam radiation (50 Gy, 2/95)	Yes (4/95)	Single (8/95)	10	Yes (6/96)
6	40/F	M-	None	None	—	Double (12/96)	23	No
7	57/F	M-	None	None	—	Double (11/97)	10	Yes (9/98)

*The age (in years) at the time of the original diagnosis is shown.

†M- denotes mucin-negative, and M+ mucin-positive.

‡Patients who did not receive conventional therapy had bilateral lung involvement at the time of the original diagnosis.

§The number of lungs transplanted was based on the number of donor lungs available.

RESULTS

Seven patients with bronchioloalveolar carcinoma received transplants of one or two cadaveric lungs (Table 1). Four patients had undergone surgical resection of the original tumor with intention to cure, but in all four patients, recurrences appeared 4 to 17 months later. The remaining three patients had stage IV disease at the time of presentation and were not candidates for conventional surgical resection. Four

of the recipients of transplants were free of disease for more than 38 months. At this writing, two of these patients (Patients 2 and 4) are alive 62 and 50 months, respectively, after transplantation and have no evidence of recurrence.

Of the four patients with a recurrence, Patient 7 had a microscopic focus in a transbronchial-biopsy specimen; the recurrence has not yet been confirmed by a more extensive surgical biopsy. Recurrences in Pa-

tients 1 and 3 were treated by resection of the involved lung tissue; both patients have no evidence of recurrent disease 18 and 4 months later, respectively. The recurrence in Patient 5 was treated by resection of the entire transplanted lung followed by a second lung transplantation. This patient died nine months later from multiple pulmonary complications, including recurrent bronchioloalveolar carcinoma within the engrafted lung. An autopsy of Patient 5 confirmed the presence of extensive bronchioloalveolar carcinoma within the transplanted lung, but microscopical analyses of the brain, liver, adrenals, kidneys, pancreas, spleen, bone marrow, breast, thyroid, heart, and a periaortic lymph node were negative for tumors.

A histologic analysis of the recurrent tumors within the transplanted lungs showed that these tumors were similar to the original tumors in the first three patients to have recurrence. Patients 3 and 5 had mucin-positive bronchioloalveolar carcinoma, and the recurrent tumors that appeared in them were also mucin-positive. In Patient 1, both the original tumor and the recurrent tumor were mucin-negative.

To study the original and recurrent tumors from Patients 1, 3, and 5, we used the polymerase chain reaction to amplify highly polymorphic dinucleotide repeats (microsatellite DNA).¹⁰ In all three patients, the microsatellite fragments found in DNA from the donor lung were clearly different from those in DNA from the original tumor. By contrast, in Patients 1 and 3, the fragments amplified in the recurrent tumors were almost identical to those in the original tumors. Although some differences between the original and recurrent tumors in Patient 5 were discernible, the recurrent tumor was clearly more similar to the original tumor than to the donor lung. These results support the notion that the recurrent bronchioloalveolar carcinoma found in the donor lungs originated in the recipients.

DISCUSSION

For four of seven patients, lung transplantation for stage IV bronchioloalveolar carcinoma limited to the lungs was followed by a lengthy period of disease-free survival. Two of the seven patients are alive with no evidence of disease 62 and 50 months after transplantation. The survival rate for patients with stage IV bronchioloalveolar carcinoma rarely exceeds five years.^{4,8} In four of the seven patients, there were recurrences in the donor lungs at various times after transplantation. Histologic and molecular analyses of these recurrent tumors in three patients strongly suggested that the recurrences arose from the original bronchioloalveolar carcinoma in the native lungs of the patients.

In all four of these cases, the recurrences were limited to the donor lungs. In the case of Patient 5, who died of pulmonary complications of the recurrent tumor, an autopsy revealed extensive intrapulmonary

bronchioloalveolar carcinoma without any microscopical evidence of extrapulmonary bronchioloalveolar carcinoma.

The site from which the recurrent bronchioloalveolar carcinoma arose within the donor lungs is unclear. Because the trachea and proximal mainstem bronchi were preserved in all of these patients as a requirement for transplantation of the donor lungs, it is possible that some bronchioloalveolar-carcinoma cells were present in these structures and were "washed" back into the transplanted lungs. If this was the case, the bronchioloalveolar-carcinoma cells must have remained dormant within the transplanted lungs for several years in Patients 1 and 3 before developing into clinically detectable disease. It is also possible that before transplantation, bronchioloalveolar-carcinoma cells disseminated to extrapulmonary sites, where they remained dormant, and later migrated back to the lung transplants. In support of this possibility, several groups have presented evidence that there are neoplastic cells in the blood or bone marrow of patients with non-small-cell lung cancer, even in early stages of the disease.¹¹⁻¹⁶

Because all the recurrences appear to have originated from microscopic disease extant at the time of transplantation, it is interesting that suppression of the cell-mediated immunity required for allograft survival did not clearly enhance the spread or growth of extrapulmonary foci of bronchioloalveolar carcinoma. Further examination of the tumor genotypes, particularly in the context of genes commonly mutated in lung cancers, may help define differences that are associated with the variable clinical course of these patients after transplantation.

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REFERENCES

- Schraufnagel D, Poloquin A, Pare JAP, Wang N-S. Differentiating bronchioloalveolar carcinoma from adenocarcinoma. *Am Rev Respir Dis* 1982; 125:74-9.
- Auerbach O, Garfinkel L. The changing pattern of lung carcinoma. *Cancer* 1991;68:1973-7.
- Barsky SH, Cameron R, Osann KE, Tomita D, Holmes EC. Rising incidence of bronchioloalveolar lung carcinoma and its unique clinicopathologic features. *Cancer* 1994;73:1163-70.
- Travis WD, Travis LB, Devesa SS. Lung cancer. *Cancer* 1995;75:Suppl: 191-202. [Erratum, *Cancer* 1995;75:2979.]
- Greco RJ, Steiner RM, Goldman S, Cotler H, Patchefsky A, Cohn HE. Bronchoalveolar cell carcinoma of the lung. *Ann Thorac Surg* 1986;41: 652-6.
- Landis SH, Murray T, Bolden S, Wingo PA. Cancer statistics, 1998. *CA Cancer J Clin* 1998;48:6-29. [Erratum, *CA Cancer J Clin* 1998;48: 192.]

7. Feldman ER, Eagan RT, Schaid DJ. Metastatic bronchioloalveolar carcinoma and metastatic adenocarcinoma of the lung: comparison of clinical manifestations, chemotherapeutic responses, and prognosis. *Mayo Clin Proc* 1992;67:27-32.
8. Grover FL, Piantadosi S. Recurrence and survival following resection of bronchioloalveolar carcinoma of the lung — the Lung Cancer Study Group experience. *Ann Surg* 1989;209:779-90.
9. Daly RC, McGregor CG. Surgical issues in lung transplantation: options, donor selection, graft preservation, and airway healing. *Mayo Clin Proc* 1997;72:79-84.
10. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989;44:388-96.
11. Cote RJ, Beattie EJ, Chaiwun B, et al. Detection of occult bone marrow micrometastases in patients with operable lung carcinoma. *Ann Surg* 1995;222:415-25.
12. Izbicki JR, Passlick B, Hosch SB, et al. Mode of spread in the early phase of lymphatic metastasis in non-small-cell lung cancer: significance of nodal micrometastasis. *J Thorac Cardiovasc Surg* 1996;112:623-30.
13. Pantel K, Izbicki J, Passlick B, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without metastases. *Lancet* 1996;347:649-53.
14. Ohgami A, Mitsudomi T, Sugio K, et al. Micrometastatic tumor cells in the bone marrow of patients with non-small-cell lung cancer. *Ann Thorac Surg* 1997;64:363-7.
15. Maruyama R, Sugio K, Mitsudomi T, Saitoh G, Ishida T, Sugimachi K. Relationship between early recurrence and micrometastases in the lymph nodes of patients with stage I non-small-cell lung cancer. *J Thorac Cardiovasc Surg* 1997;114:535-43.
16. Peck K, Sher Y-P, Shih J-Y, Roffler SR, Wu C-W, Yang P-C. Detection and quantitation of circulating cancer cells in the peripheral blood of lung cancer patients. *Cancer Res* 1998;58:2761-5.