THE MOUNT SINAI JOURNAL OF MEDICINE
Vol. 56, No. 2, March 1989
Printed in U.S.A.

Activity of pp60$^{c\text{-}src}$ Protein Kinase in Human Breast Cancer

STEVEN LEHRER, M.D., JOYCE O'SHAUGHNESSY, M.D., HEE KYUNG SONG, M.D., EVA LEVINE, M.S., PENNY SAVORETTI, RN, JACK DALTON, M.D., ROBERTO LIPSZTEIN, M.D., SHALOM KALNICKI, M.D., AND WILLIAM D. BLOOMER, M.D.

Abstract

Because there is elevation of pp60$^{c\text{-}src}$ activity in breast carcinoma tissue, we analyzed primary breast cancer tissue samples from 30 women to determine whether pp60$^{c\text{-}src}$ activity correlated with specific clinical parameters. We found that tumors with a progesterone receptor had higher pp60$^{c\text{-}src}$ activity than tumors without such a receptor. But there was no association of pp60$^{c\text{-}src}$ activity with the presence of an estrogen receptor or of nodes, or with menopausal status or age. The function of pp60$^{c\text{-}src}$ in normal cells and in breast cancer is unknown, as is the significance of our finding of an association of elevated pp60$^{c\text{-}src}$ activity and the presence of progesterone receptors.

The *src* oncogene is a gene carried by an RNA virus, a retrovirus. When the retrovirus infects an animal cell, its RNA is transcribed into DNA. Responsible for rapid oncogenesis, *v-src* was first identified in the early 1970s in Rous sarcoma virus, which causes cancer in chickens. It was named *src* for sarcoma. The protein encoded by *v-src* is designated as pp60 because its molecular weight is 60,000 d (1).

The *src* oncogene is not truly a viral gene. Instead, it is a nearly exact copy of a gene found in all chicken cells. The normal proto-oncogene was picked up by a weakly oncogenic retrovirus in the course of infection, and in the process it was transformed into a cancer gene (2).

Numerous oncogenes have been isolated from retroviruses that cause carcinoma, sarcoma, leukemia, or lymphoma in chickens, other birds, rats, mice, cats, and monkeys. All of these oncogenes are closely related to a normal gene in the host animal and encode an oncogenic transforming protein similar to a normal protein.

The *src* protein, pp60, is an enzyme that catalyzes the addition of a phosphate molecule to other proteins. Such enzymes are called *protein kinases,* from the Greek *kinein,* "to move." They transfer the energy-rich terminal phosphate group of adenosine triphosphate (ATP), the cell's major energy carrier, to the protein being modified. This phosphorylation process is an important modulator of protein function. Both pp60$^{v\text{-}src}$ and its cellular counterpart pp60$^{c\text{-}src}$ phosphorylate the amino acid tyrosine (3).

The most extensively studied of the tyrosine-specific protein kinases is pp60$^{c\text{-}src}$. This enzyme, encoded by the *c-src* gene, is the protein produced by the proto- (or cellular) oncogene *c-src*. It is closely related, but not identical, to pp60$^{v\text{-}src}$, the enzyme encoded by the Rous sarcoma virus transforming gene *v-src,* that is, the *src* oncogene (4).

Rosen et al have estimated the abundance of the *c-src* protein in human tumor tissue and human tumor cell lines (4). They found that pp60$^{c\text{-}src}$ kinase activity in breast carcinoma tissue was significantly elevated when compared to normal breast tissue. However, not all breast

From the Department of Radiotherapy, Mount Sinai School of Medicine (CUNY), New York, and the Laboratory of Tumor Virus Biology, National Institutes of Health, Bethesda, Maryland (J O'S). Address reprint requests to Steven Lehrer, MD, Box 1236, Radiotherapy Department, Mount Sinai Medical Center, One Gustave L. Levy Place, New York, NY 10029.

TABLE
Clinical Data, Tumor Hormone Receptor Status, and Tumor pp60$^{c\text{-}src}$ Activity in 30 Women

Clinical characteristics	No. Cases	pp60 (mean ± SD)	Significance
Nodal involvement*			
Negative	17	2.1 ± 1.2	
Positive	8	1.9 ± 2.1	*p* NS
Menopausal status*			
Premenopausal	13	2.0 ± 1.2	
Postmenopausal	13	1.7 ± 1.2	*p* NS
Age			
52 ± 16 yr	3	0	
55 ± 12 yr	9	1	f = 0.22‡
52 ± 14 yr	8	2	
52 ± 9 yr	6	3	
58 ± 12 yr	4	4	*p* NS
Estrogen receptor†			
Negative	17	1.7 ± 1.4	
Positive	13	2.2 ± 1.1	*p* NS
Progesterone receptor†			
Negative	10	1.3 ± 1.05	$t = 2.17$
Positive	20	2.3 ± 1.2	$p < 0.05$ 2-tailed

* Even though 30 women participated in this study, data on nodal involvement were available in only 25 cases, and data on menopausal status in only 26 cases.

† Although there was a significant difference between pp60 in progesterone-receptor positive and negative cases, the difference was not significant when estrogen and progesterone receptor data were combined and analyzed by one-way analysis of variance (f = 1.79).

‡ One-way ANOVA.

carcinoma cell lines had high levels of pp60$^{c\text{-}src}$ kinase activity.

In the study reported here, primary breast cancer tissue samples were analyzed to determine whether pp60$^{c\text{-}src}$ activity correlated with specific clinical parameters, including the presence of an estrogen receptor (ER) or progesterone receptor (PR) in the tumor.

Materials and Methods

Breast cancer tissue, removed from 30 women undergoing lumpectomies or mastectomies, was analyzed. All tumor tissue came from the primary lesion; none was taken from nodes.

The pp60$^{c\text{-}src}$ activity in the tissue specimens was measured by a sensitive in vitro immune complex protein kinase assay of cytoplasmic extracts of the specimens. The method has been described previously (4). Two monoclonal antibodies to pp60^{src} protein were used to ensure specificity. These antibodies were derived from mouse myeloma cells fused with spleen cells of mice immunized with purified pp60^{src}, produced from bacterial recombinants (5). Phosphotyrosine was identified as the kinase product.

Estrogen and progesterone receptor concentrations in tumor tissue were determined with a standard dextran-coated charcoal-binding assay (6). A receptor was considered to be present if there was greater than 5 fmol/mg cytosol protein of bound hormone.

The degree of pp60$^{c\text{-}src}$ kinase activity was determined by assaying the level of autophosphorylation and exogenous substrate phosphorylation in the presence of gamma ^{32}P-ATP. The degree of kinase activity was scored as 0 to 4, to allow for statistical correlation with other numerical parameters, 0 representing no detectable kinase activity, 1 representing normal breast tissue or fibroblast levels, and 2, 3, and 4 representing approximately fivefold, tenfold, and twentyfold increases in kinase activity respectively, as measured by scanning densitometry of the autoradiographs.

Results

Tumors with a progesterone receptor had higher pp60$^{c\text{-}src}$ activity than tumors without a receptor ($p < 0.05$, two-tailed). One-way ANOVA of ER and PR data was not significant (f = 1.79); also, there was no significant linear correlation between PR and pp60$^{c\text{-}src}$ levels ($r = -0.26$). (Table)

There was no significant relationship of pp60$^{c\text{-}src}$ activity to menopausal status or the presence of axillary lymph nodes pathologically positive for tumor. Women with maximal pp60$^{c\text{-}src}$ activity (+4) were slightly older than women with no detectable activity; but one-way analysis of variance revealed that overall, the age difference was insignificant. Moreover, the presence or absence of an estrogen receptor in the tumor tissue did not significantly correlate with pp60$^{c\text{-}src}$ activity.

Discussion

The progesterone receptor is at least as valuable as the estrogen receptor for predicting outcome in breast cancer patients (7). Indeed, the progesterone receptor may be the second most critical factor, after the number of positive nodes, in predicting disease-free survival. In addition, the presence of a progesterone receptor suggests a favorable response to tamoxifen therapy in women with both early and advanced breast cancer (7). Only amplification of the recently discovered HER-2/*neu* oncogene may rival the predictive power of the progesterone receptor (8).

The significance of our preliminary finding of an association between elevated pp60$^{c\text{-}src}$ activity and the presence of progesterone receptors in human breast cancer is unknown. The function of pp60$^{c\text{-}src}$ in normal cells and in breast cancer is also not known.

It is of interest that we found no significant association of pp60$^{c\text{-}src}$ activity with age or menopausal status. Studies of familial breast cancer, no doubt induced by one or more genes, show that this disorder occurs in young women who are predominantly premenopausal (9). We conclude, therefore, that there is probably no relationship between the *c-src* gene and familial breast cancer.

If more can be learned about this subject, our understanding of the pathogenesis of breast cancer might be broadened.

Acknowledgments

We thank Dr. D. Pertsemlidis for his help during the performance of this study.

References

1. Collett MS, Erikson RL. Protein kinase activity associated with the avian sarcoma virus gene product. Proc Nat Acad Sci 1978; 75(4):2021–2024.
2. Bishop JM, Varmus H. Functions and origins of retroviral transforming genes. In: Weiss R, Teich N, Varmus H, Coffin J, eds. The molecular biology of tumor viruses. Cold Spring Harbor: Cold Spring Harbor Press, 1982, 999–1108.
3. Hunter T. The proteins of oncogenes. Sci Amer 1984; 251(2):70–79.
4. Rosen N, Bolen JB, Schwartz AM, Cohen P, De Seau V, Israel MA. Analysis of pp60$^{c\text{-}src}$ protein kinase activity in human tumor cell lines and tissues. J Biol Chem 1986; 261(29):13754–13759.
5. Lipsich LA, Lewis AJ, Brugge J. Isolation of monoclonal antibodies that recognize the transforming proteins of avian sarcoma viruses. J Virol 1983; 48:352–360.
6. Bayard F, Damilano S, Robel P, Baulieu EE. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. J Clin Endocrinol Metab 1978; 46:635–648.
7. McGuire WL, Clark GM. Role of progesterone receptors in breast cancer. CA 1986; 36(5):302–310.
8. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235:177–181.
9. Vorherr H. Breast cancer: epidemiology, endocrinology, biochemistry, and pathobiology. Baltimore-Munich: Urban & Schwarzenberg, 1980, 62–64.

Submitted for publication May 1988.