ETV6-NTRK3—Trk-ing the primary event in human secretory breast cancer

In this issue of *Cancer Cell*, Tognon et al. (2002) propose a single chromosomal translocation as the cause of a particularly troubling form of breast cancer, secretory carcinoma. They challenge widely held beliefs concerning breast carcinogenesis as well as beliefs concerning the absolute association of specific fusion genes with specific tumor types. Their data highlight the role of Trk signaling in breast cancer and also suggest a target for drug development.

Secretory carcinoma is a rare form of breast cancer, aptly named for the eosinophilic material that accumulates in intracellular vacuoles and intercellular lacunae (Page, 1987). It is a rare tumor, accounting for fewer than 1% of all breast cancers, but it is particularly troublesome because it occurs in patients as young as three years old, and frequently requires mastectomy and chemotherapy for treatment. Mastectomy in prepubescent females causes complex psychosocial difficulties in adolescence. Experience dictates that most early onset breast cancers are caused by mutations in genes related to DNA repair. We were unable to identify mutations in *p53*, *BRCA1*, or *BRCA2* in secretory carcinomas from two patients, age five and six, we recently cared for (unpublished data). In fact, while p53 is the most commonly altered molecule in infiltrating ductal carcinoma, it is curiously unaffected in secretory breast cancer (Maitra et al., 1999).

Tognon and colleagues have detected the *ETV6-NTRK3* fusion gene in 12 (92%) of 13 secretory breast carcinomas, but only 1 (2%) of 50 infiltrating ductal carcinomas. This gene is the product of a t(12;15)(p13;q25) translocation that fuses the dimerization domain of a transcriptional regulator (ETV6) with a membrane receptor tyrosine kinase (NTRK3). Prior work confirms that the ETV6-NTRK3 fusion protein can activate the Ras-Mek1 and PI3K-Akt pathways which are important for breast cell proliferation and survival. The methods employed in the current study are unassailable, including fluorescence in situ hybridization to demonstrate the *ETV6-NTRK3* fusion gene at the DNA level, sequencing of RT-PCR products to demonstrate *ETV6-NTRK3* expression at the mRNA level, and immunoprecipitation to confirm ETV6-NTRK3 expression at the protein level. In addition, retroviral transduction of the *ETV6-NTRK3* gene into two murine mammary epithelial cell lines resulted in transformation and tumorigenesis in nude mice with preservation of the original phenotype.

The suggestion that an alteration in a single membrane tyrosine kinase receptor is sufficient to transform mammary epithelial cells should capture our attention, but the fact that it is the ETV6-NTRK3 fusion protein that does it should prompt a double take. The *ETV6-NTRK3* fusion gene is associated with infant fibrosarcoma and congenital mesoblastic nephroma, two morphologically similar pediatric mesenchymal tumors with no epithelial features (Figure 1). The occurrence of the same fusion gene in an epithelial malignancy violates the notion that specific fusion genes are only associated with specific tumor types (Ladanyi and Bridge, 2000). In fact, fusion genes are the basis of a growing molecular taxonomy for soft tissue tumors, and reports of the "wrong" fusion gene occurring in association with a particular histology prompted one investigator to write, "Unexpected positive results often attract more attention and often lead to publication of case reports. The appearance of papers reporting unexpected PCR results that subsequently prove to be irreproducible has become an increasingly frequent event in recent years" (Ladanyi and Bridge, 2000). Given the rigorous evaluation of the *ETV6-NTRK3* fusion

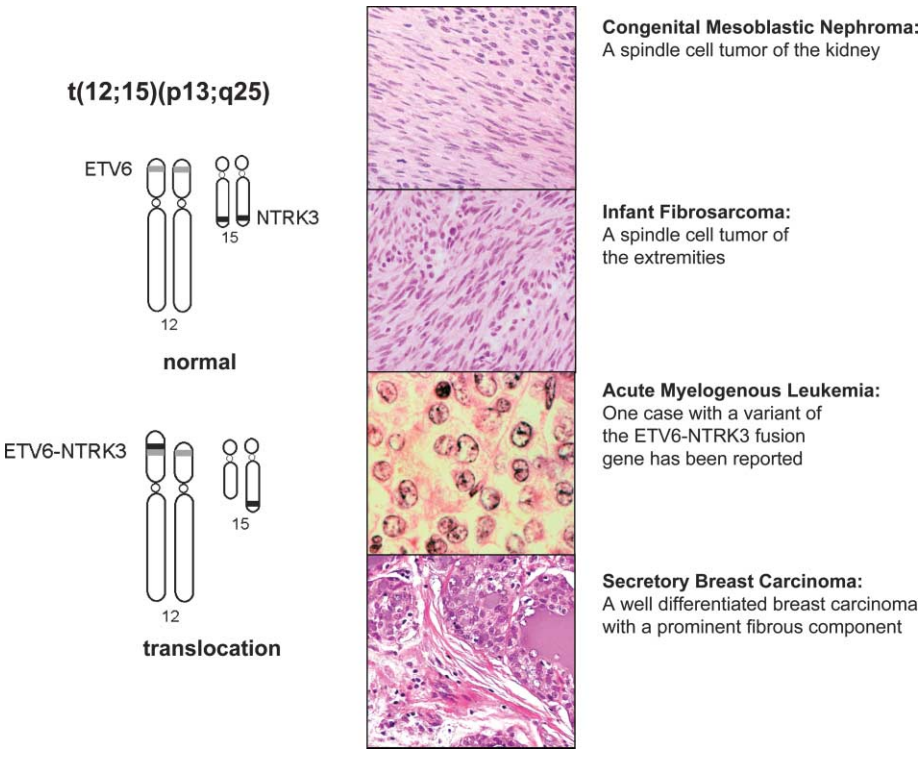

Figure 1. The t(12;15)(p13;q25) translocation fuses the *ETV6* and *NTRK3* genes presumably resulting in constitutive expression of NTRK3 tyrosine kinase activity

The association of this fusion gene with malignancies as morphologically distinct as congenital mesoblastic nephroma and secretory breast cancer is notable. One case of acute myelogenous leukemia with a variant of the *ETV6-NTK3* fusion gene has been reported (Eguchi et al., 1999).

gene in secretory breast carcinoma described by Tognon and coworkers, it is highly unlikely that these results will be "irreproducible." It must be remembered, however, that the majority of fusion genes contributing to the molecular taxonomy of soft tissue tumors are chimeric transcription factors and would be expected to influence differentiation according to the types of genes they activate. As a receptor tyrosine kinase capable of activating the Ras-Mek1 and PI3K-Akt pathways, which are implicated in many tumor types, *ETV6-NTRK3* appears to be a more promiscuous oncogene that transforms without interfering with differentiation programs. This is reminiscent of fusion genes containing the ALK tyrosine kinase which have been associated with diseases as morphologically distinct as inflammatory myofibroblastic tumors and anaplastic large cell lymphoma (Lawrence et al., 2000).

Beyond the obvious implications for understanding carcinogenesis, fusion genes that lead to constitutive activation of membrane receptor tyrosine kinases are of interest for their potential as therapeutic targets. The drug imatinib mesylate (Gleevec) was identified by screening chemical libraries for compounds with tyrosine kinase inhibitory activity (Druker, 2002). The most promising candidate was structurally modified based on knowledge of structure and activity and then optimized against the platelet-derived growth factor receptor (PDGFR). The resulting drug, which is specific for the Abl tyrosine kinase (of BCR-ABL fame), PDGFR and c-kit, has revolutionized the treatment of chronic myelogenous leukemia. Given the involvement of Trk signaling in malignancies as diverse as congenital mesoblastic nephroma, infant fibrosarcoma, melanoma, medullary thyroid cancer, pancreatic carcinoma, prostate cancer, and breast carcinoma, it would seem reasonable to screen chemical libraries for compounds capable of specifically inhibiting Trk signaling with the intent of developing targeted therapies for these malignancies.

Tognon et al. have unequivocally demonstrated involvement of the *ETV6-NTRK3* fusion gene in secretory breast carcinoma, but several questions remain to be answered. Expression of the *ETV6-NTRK3* fusion gene in pediatric soft tissue tumors is nearly always accompanied by chromosome 11p abnormalities including partial duplications or chromosome 11 multiploidy, both of which affect the insulin-like growth factor-2 (*IGF-2*) locus at 11p15.5 resulting in IGF-2 overexpression (Knezevich et al., 1998) (Watanabe et al., 2002). Is this also the case for secretory breast carcinoma? In addition, juvenile papillomatosis is a benign multicystic neoplasm of the breast characterized by ductal papillomatosis and proliferation of atypical apocrine cells. Because juvenile papillomatosis has been reported in association with secretory carcinoma (and other breast cancers) (Rosen et al., 1982), it would be interesting to know whether the *ETV6-NTRK3* fusion gene can be identified in this benign lesion. This is not necessarily to suggest that secretory carcinoma arises from juvenile papillomatosis, but that the two conditions may represent alternative differentiation pathways for stem cells expressing the *ETV6-NTRK3* fusion gene. Finally, it has not been shown unequivocally that it is the epithelial cells of the secretory carcinomas that express *ETV6-NTRK3*. Finding the fusion gene in the stromal cells of this neoplasm would have intriguing implications for stromal-epithelial interaction during carcinogenesis or for epithelial-to-mesenchymal transition during progression.

This study has raised many intriguing questions. It appears that a more thorough evaluation of the Trk signaling pathway in breast cancer is in order, as is a search for other tumor types expressing the *ETV6-NTRK3* fusion gene. Though congenital mesoblastic nephroma, infant fibrosarcoma, and secretory breast carcinoma rarely metastasize, systemic therapy is sometimes required for cure. Given the high toxicity of conventional cytotoxic chemotherapy in very young infants, a specific NTRK3 tyrosine kinase inhibitor would be a welcome addition to the therapeutic armamentarium.

David M. Euhus,[1,4] Charles F. Timmons,[2] and Gail E. Tomlinson[3]

[1]Department of Surgery
[2]Department of Pathology
[3]Department of Pediatrics
University of Texas
Southwestern Medical Center at Dallas
5323 Harry Hines Boulevard
Dallas, Texas 75390
[4]E-mail: david.euhus@utsouthwestern.edu

Selected reading

Druker, B.J. (2002). Trends Mol. Med. *8*, S14–S18.

Eguchi, M., Eguchi-Ishimae, M., Tojo, A. Morishita, K., Suzuki, K., Sato, Y., Kudoh, S., Tanaka, K., Setoyama, M., Nagamura, F., Asano, S., and Kamada, N. (1999). Blood *93*, 1355–1363.

Knezevich, S.R., Garnett, M.J., Pysher, T.J., Beckwith, J.B., Grundy, P.E., and Sorensen, P.H.B. (1998). Cancer Res. *58*, 5046–5048.

Ladanyi, M., and Bridge, J.A. (2000). Hum. Pathol. *31*, 532–538.

Lawrence, B., Perez-Atayde, A., Hibbard, M.K., Rubin, B.P., Dal Cin, P., Pinkus, J.L., Pinkus, G.S., Xiao, S., Yi, E.S., Fletcher, C.D., and Fletcher, J.A. (2000). Am. J. Pathol. *157*, 377–384.

Maitra, A., Tavassoli, F.A., Albores-Saavedra, J., Behrens, C., Wistuba, I., Bryant, D., Weinberg, A.G., Rogers, B.L., Saboorian, M.H., and Gazdar, A.F. (1999). Hum. Pathol. *30*, 1435–1440.

Page, D.L. (1987). In Diagnostic Histopathology of the Breast (Edinburgh: Churchill Livingston), pp. 236–239.

Rosen, P.P., Lyngholm, B., Kinne, D.W., and Beattie, E.J. (1982). Cancer *49*, 2591–2595.

Tognon, C., Knezevich, S.R., Huntsman, D., Roskelley, C.D., Melnyk, N., Mathers, J.A., Becker, L., Carneiro, F., MacPherson, N., Horsman, D., et al. (2002). Cancer Cell *2*, this issue, 367–376.

Watanabe, N., Kobayashi, H., Hirama, T., Kikuta, A., Koizumi, S., Tsuru, T., and Kaneko, Y. (2002). Cancer Genet. Cytogenet. *136*, 10–16.