MOLECULAR AND CELLULAR BIOLOGY, Mar. 1986, p. 955–958
0270-7306/86/030955-04$02.00/0

Vol. 6, No. 3

Localization of a Novel v-*erb*B-Related Gene, c-*erb*B-2, on Human Chromosome 17 and Its Amplification in a Gastric Cancer Cell Line

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Received 17 September 1985/Accepted 3 December 1985

The c-*erb*B-2 gene is a v-*erb*B-related proto-oncogene which is distinct from the gene encoding the epidermal growth factor receptor. By using two independent methods, hybridization of both sorted chromosomes and metaphase spreads with cloned c-*erb*B-2 DNA, we mapped the c-*erb*B-2 locus on human chromosome 17 at q21, a specific breakpoint observed in a translocation associated with acute promyelocytic leukemia. Furthermore, we observed amplification and elevated expression of the c-*erb*B-2 gene in the MKN-7 gastric cancer cell line. These data suggest possible involvement of the c-*erb*B-2 gene in human cancer.

A number of cellular counterparts to the retroviral oncogenes have been identified and localized on specific chromosomes. The locations of several cellular oncogenes correspond well to breakpoints of chromosomal translocations found in various cancers. For example, the c-*myc* gene on chromosome 8 is involved in translocations between chromosome 8 and one of the chromosomes—2, 14, or 22—that carries an immunoglobulin gene (5, 9, 12, 22). The resulting alteration in c-*myc* expression is suspected to be causally related to tumorigenesis (14).

An avian erythroblastosis virus H strain contains an oncogene, v-*erb*B, that replaces the *env* gene of an avian leukosis virus (25). The nucleotide sequence analysis of the cloned v-*erb*B DNA and human epidermal growth factor (EGF) receptor cDNA clones revealed that the v-*erb*B protein corresponds to the carboxyl half of the human EGF receptor, including the membrane-spanning domain (23, 24, 26). This strongly suggests that the 3′ half of the chicken EGF receptor gene was transduced into the H strain of avian erythroblastosis virus. In addition to the EGF receptor gene, we found another v-*erb*B-related gene, c-*erb*B-2, in the human genome. The c-*erb*B-2 gene is apparently distinct from the EGF receptor gene, since transcripts of the two genes differ from each other in length and because the amino acid sequence predicted from the nucleotide sequence of cloned c-*erb*B-2 gene is very similar to the corresponding region of the EGF receptor (17). Recently, the *neu* oncogene, active in a series of rat neuroblastoma (19), was found to be an *erb*B-related gene encoding an EGF receptor-like protein (C. I. Bargmann, M.-C. Huang, and R. A. Weinberg, Nature [London], in press). Comparison of the nucleotide sequences and the deduced amino acid sequences of human c-*erb*B-2 (T. Yamamoto, S. Ikawa, T. Akiyama, K. Semba, N. Nomura, N. Miyajima, T. Saito, and K. Toyoshima, Nature [London], in press) and rat *neu* (Bargmann et al., in press) revealed a strong similarity between the two genes, which suggests that they are in fact the same gene.

Metaphase chromosomes were prepared from two cell lines, GM2324 and GM3197, which were provided by the Human Genetic Mutant Cell Repository and then sorted (Institute for Medical Research, Camden, N.J.), into nine fractions using a fluorescence-activated cell sorter as described previously (20, 28). DNA samples were prepared from each fraction of the sorted chromosomes (7) and analyzed by Southern hybridization (21) using a DNA probe of a 440-base-pair (bp) *Kpn*I-*Xba*I restriction fragment (KX-DNA) generated from the c-*erb*B-2 genomic clone λ107. With the GM2324 cell line, a positive signal was observed for a fraction that corresponded mainly to chromosomes 16, 17, and 18 (data not shown). Another human lymphoblast culture, GM3197, carries the reciprocal translocation, t(17;22), producing derivative chromosomes (17;22 and 22q$^-$) that are different in size from the normal homologs (6). Analysis of this cell line revealed two positive signals, one in a fraction that contains normal chromosome 17 and one in a fraction that contains the derivative chromosome 17;22 (data not shown). These results indicate that the c-*erb*B-2 gene is located on human chromosome 17.

To localize the c-*erb*B-2 gene more precisely, we performed in situ hybridization experiments on chromosome spreads prepared from phytohemagglutinin-stimulated peripheral blood cultures (3, 29). The probe used for this experiment was ^{3}H-labeled pCER217 plasmid DNA, which is a c-*erb*B-2 cDNA clone containing a 2.7-kbp insert in the Okayama-Berg vector (15). Analysis of 85 metaphase cells revealed that 23.5% (20 of 85) of the silver grains were located on chromosome 17. Of these 20 grains, 15 (75%) were located on band q21-q22, and 11 grains in particular were in the region 17q21.1-21.3 (Fig. 1). A human version of the *neu* oncogene was recently mapped on human chromosome 17 at q21 (16). Since translocation between chromosomes 15 and 17, t(15;17) (q23;q21), is associated with acute promyelocytic leukemia (APL) (13), we examined DNA from seven cases of APL for the possible involvement of the c-*erb*B-2 gene in this leukemia. Using the ^{32}P-labeled fragment prepared from pCER217 as a probe, we observed no sign of rearrangement of the c-*erb*B-2 gene by Southern hybridization analysis (data not shown). Recently, the *p53* gene was also mapped to human chromosome 17 at bands 17q21-q22. Although rearrangements of the *p53* gene were not observed on Southern blotting of DNAs from APL cells

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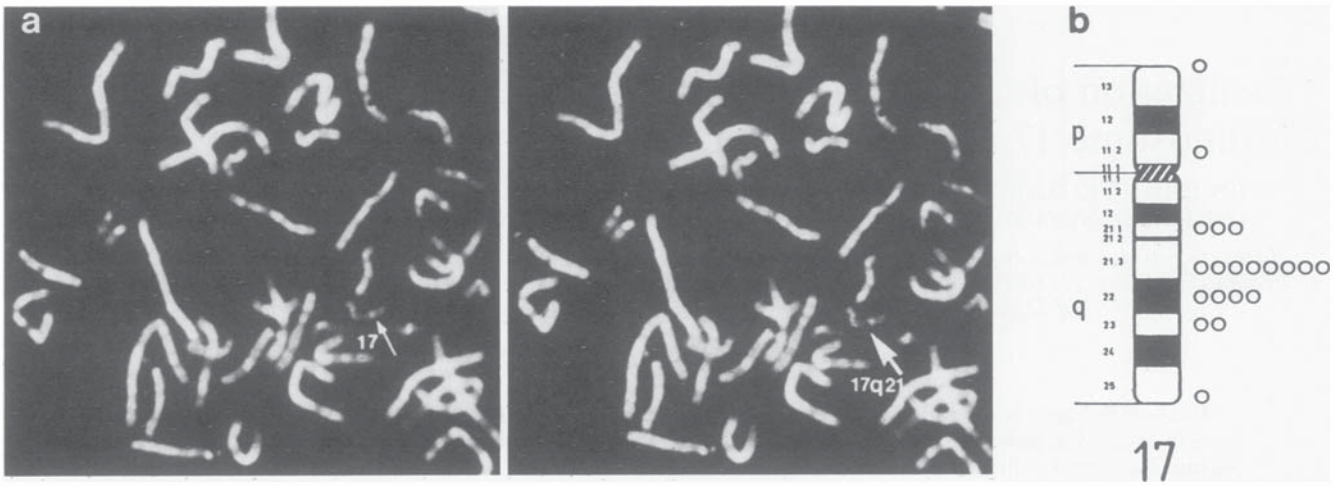

FIG. 1. Localization of the c-*erb*B-2 gene by in situ hybridization. (a) Photograph of a lymphocyte metaphase spread hybridized with the c-*erb*B-2 probe nick translated with [^{3}H]dCTP (30 Ci/mmol) and [^{3}H]dTTP (48 Ci/mmol). The specific activity of the probe was 3×10^7 cpm/μg of DNA. The chromosomal DNA was denatured on slides in 70% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 70°C for 2 min and then hybridized in a solution of 50% formamide–2× SSC–40 mM sodium phosphate (pH 7.0)–10% dextran sulfate–denatured salmon sperm DNA (100 μg/ml)–1× Denhardt solution for 16 h at 40°C. After hybridization, the slides were rinsed for 10 min twice in 50% formamide–2× SSC at 40°C and then several times in 0.2× SSC at 37°C. Autoradiography was performed using half-strength Sakura NR-M2 emulsion (Konishiroku, Tokyo) for 3 weeks at 4°C. Chromosomes were Q banded using the double-staining method with quinacrine-mustard and Hoechst 33258 (27) and analyzed under a fluorescence microscope (left). Silver grains were detected by visible light and were identified on Q-banded chromosomes (right). (b) Distribution of 20 grains over chromosome 17.

with t(15;17), translocation of the *p53* gene to chromosome 15 was observed in three of three APL cases tested (8) by in situ hybridization. Thus, further analysis of APL cells with t(15;17), which include in situ hybridization on chromosome spread using c-*erb*B-2-specific DNA probes, is anticipated.

Previously, we found that the c-*erb*B-2 gene is amplified in an adenocarcinoma of the salivary gland, although we could

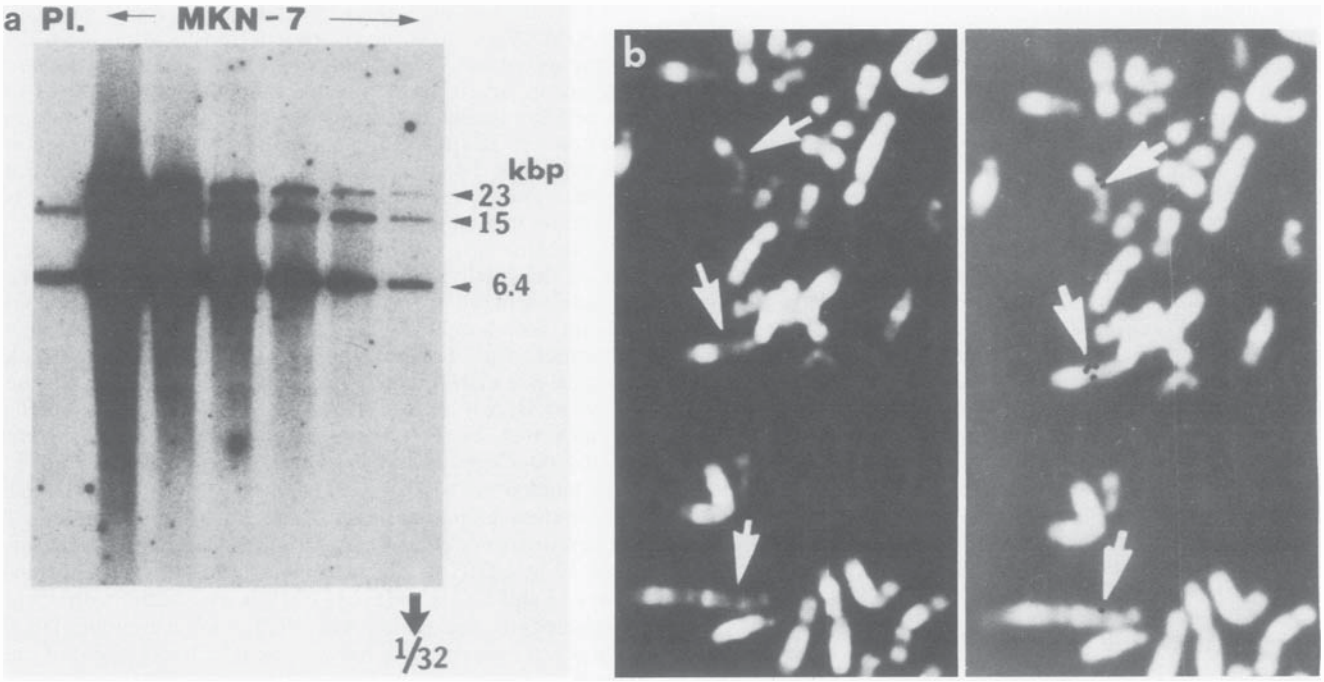

FIG. 2. Amplification and translocation of the c-*erb*B-2 gene in a gastric cancer cell line, MKN-7. (a) Amplification of the c-*erb*B-2 gene. High-molecular-weight DNAs were prepared from MKN-7 and human placental cells and digested with restriction endonuclease *Eco*RI. A nitrocellulose filter containing the *Eco*RI digests was probed with ^{32}P-labeled KX-DNA (specific activity; 10^8 cpm/μg of DNA). Hybridization was carried out in a stringent condition (17). The filter contained, in lanes from left to right, placental DNA (10 μg) and MKN-7 DNA (10, 5, 2.5, 1.25, 5/8, and 5/16 μg). (b) Translocation of the c-*erb*B-2 gene. Metaphase spread was prepared from MKN-7 cells (left) and hybridized with ^{3}H-labeled pCER217 DNA (right) as described in the legend to Fig. 1. Arrows indicate location of the c-*erb*B-2 gene on marker chromosomes.

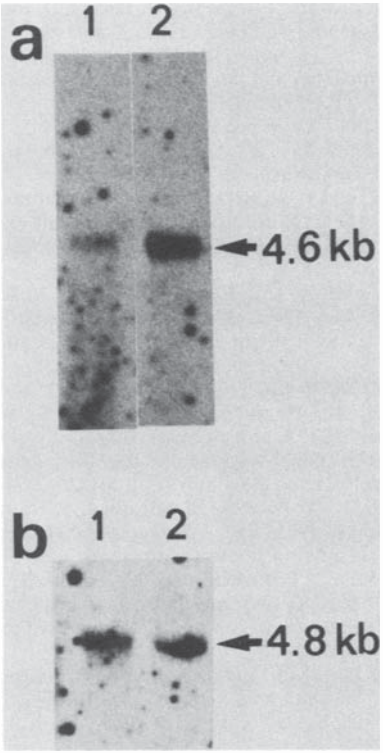

FIG. 3. Elevated expression of the c-*erb*B-2 gene in MKN-7 cells. Nitrocellulose filters containing poly(A)$^+$ RNA (2 μg) of MKN-7 cells (lane 2) and placental cells (lane 1) were hybridized with ^{32}P-labeled KX-DNA (panel a) under stringent conditions (17) or with ^{32}P-labeled v-*yes* probe (panel b) prepared from recombinant plasmid pYS2 (18) in a relaxed condition (17). DNA probes were labeled with [α-^{32}P]dCTP by nick translation to a specific activity of 1×10^8 to 2×10^8 cpm/μg of DNA.

not examine the level of RNA transcripts due to the limited amount of tissue (17). To examine the possible involvement of the c-*erb*B-2 gene in other types of tumors, we examined the DNA of 30 human cancerous cell lines for the altered structures of this gene, i.e., by gene amplification or rearrangement by Southern hybridization with the ^{32}P-labeled KX-DNA. An MKN-7 cell line established from an adenocarcinoma of the stomach was found to contain an elevated copy number of the c-*erb*B-2 gene. The degree of amplification was estimated by serial dilution of MKN-7 DNA to be about 30-fold relative to placental cell DNA (Fig. 2a). We also observed an additional *Eco*RI fragment of about 23-kbp that is specific to MKN-7 cells. This sequence is amplified to the same extent as are the other two *Eco*RI fragments. In situ hybridization analysis of MKN-7 metaphase spreads showed that the c-*erb*B-2 gene is located on at least two marker chromosomes other than chromosome 17 and that the grains were clustered (average, 4 to 5 grains) on a marker chromosome (Fig. 2b). Therefore, we assume that chromosomal translocation in MKN-7 could lead to the fusion of a part of the c-*erb*B-2 gene to an unidentified DNA, which resulted in the generation of the 23-kbp *Eco*RI fragment.

Poly(A)$^+$ RNAs were prepared from MKN-7 and placental cells. Northern hybridization of these RNAs with ^{32}P-labeled KX-DNA probe showed that an increased amount of c-*erb*B-2 mRNA (4.6 kb), in proportion to the degree of the gene amplification, is synthesized in MKN-7 cells relative to placental cells, whereas the 4.8-kb *yes* mRNA in MKN-7 remains at the same level as in placental cells (Fig. 3).

There is growing evidence that gene amplification or elevated expression of proto-oncogenes may play a role at some stage during the neoplastic progress of certain tumors. The EGF receptor gene proto-*erb*B is frequently amplified and overexpressed in glioblastomas (10, 11) and squamous cell carcinomas (2; T. Yamamoto, N. Kamata, H. Kawano, S. Shimizu, T. Kuroki, K. Toyoshima, K. Rikimaru, N. Nomura, R. Ishizaki, I. Pastan, S. Gamou, and N. Shimizu, Cancer Res., in press). Amplification of the N-*myc* gene correlates with stage III and IV neuroblastomas (1). An elevated copy number of the c-*erb*B-2 gene was found in MKN-7 cells, in a primary adenocarcinoma of the salivary gland (17), and in a primary mammary tumor (4), suggesting a possible role of the c-*erb*B-2 gene in transforming epithelial cells or in the malignancy of transformed epithelial cells.

We thank H. Kawano for excellent technical assistance and S. Sasaki for help in preparing the manuscript. We also thank G. Merlino (National Cancer Institute) for his critical reading of the manuscript.

LITERATURE CITED

1. **Brodeur, G., C. Seeger, M. Schwab, H. E. Varmus, and J. M. Bishop.** 1984. Amplification of N-*myc* in untreated human neuroblastomas correlates with advanced disease stage. Science **224**:1121–1124.
2. **Cowley, G., J. A. Smith, B. Gusterson, F. Hendler, and B. Ozanne.** 1984. The amount of EGF receptor is elevated on squamous cell carcinomas, p. 5–10. *In* J. Feramisco, B. Ozanne, and C. Stiles (ed.), Cancer cells. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Harper, M. E., and G. F. Sanders.** 1981. Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. Chromosoma **83**:431–439.
4. **King, C. R., M. H. Kraus, and S. A. Aaronson.** 1985. Amplification of a novel v-*erb*B-related gene in a human mammary carcinoma. Science **229**:974–976.
5. **Kirsch, I. R., C. C. Morton, K. Nakahara, and P. Leder.** 1982. Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. Science **216**: 301–303.
6. **Krumlauf, R., M. Jeanpierre, and B. D. Young.** 1982. Construction and characterization of genomic libraries from specific human chromosomes. Proc. Natl. Acad. Sci. USA **79**: 2971–2975.
7. **Kunkel, L. M., U. Tantravahi, M. Eisenhard, and S. A. Latt.** 1982. Regional localization on the human X of DNA segments cloned from flow sorted chromosomes. Nucleic Acids Res. **10**:1557–1578.
8. **Le Beau, M. M., C. A. Wastbrook, M. O. Diaz, J. D. Rowley, and M. Oren.** 1985. Translocation of the *p53* gene in t(15;17) in acute promyelocytic leukemia. Nature (London) **316**:826–828.
9. **Leder, P., J. Battey, G. Lenoir, C. Moulding, W. Murphy, H. Potter, T. Stewart, and R. Tanb.** 1983. Translocations among antibody genes in human cancer. Science **222**:765–771.
10. **Libermann, T. A., H. R. Nubaum, N. Razon, R. Kris, I. Lax, H. Soreq, N. Whittle, M. D. Waterfield, A. Ullrich, and J. Schlessinger.** 1985. Amplification, enhanced expression and possible rearrangement of the EGF receptor gene in primary human brain tumors of glial origin. Nature (London) **313**:144–147.
11. **Libermann, T. A., N. Razon, A. D. Bartal, Y. Yarden, J. Schlessinger, and H. Soreq.** 1984. Expression of epidermal growth factor receptors in human brain tumors. Cancer Res. **44**:753–760.
12. **Malcolm, S., P. Barton, C. Marphy, M. A. Ferguson-Smith, D. L. Bentley, and T. H. Rabbitts.** 1982. Localization of human immunoglobulin light κ chain variable region genes to the short arm of chromosome 2 by *in situ* hybridization. Proc. Natl. Acad.

Sci. USA **79**:4957–4961.
13. **Mulvihill, J. J., and P. Madigan.** 1984. Neoplasia of man (*Homo sapiens*), p. 447–449. *In* S. J. O'Brien (ed.), Genetic maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
14. **Nishikura, K., A. ar-Rushdi, J. Erikson, E. DeJesus, D. Dugan, and C. M. Croce.** 1984. Repression of rearranged μ gene and translocated c-*myc* in mouse 3T3 cells × Burkitt lymphoma cell hybrids. Science **224**:399–402.
15. **Okayama, H., and P. Berg.** 1982. High efficiency cloning of full-length cDNA. Mol. Cell. Biol. **2**:161–170.
16. **Schechter, A. L., M.-C. Hung, L. Vaidyanathan, R. A. Weinberg, T. L. Yang-Feng, U. Francke, A. Ullrich, and L. Coussens.** 1985. The *neu* gene: an *erb*B-homologous gene distinct from and unlinked to the gene encoding the EGF receptor. Science **229**:976–978.
17. **Semba, K., N. Kamata, K. Toyoshima, and T. Yamamoto.** 1985. A v-*erb*B-related protooncogene, c-*erb*B-2, is distinct from the c-*erb*B-1/EGF receptor gene and is amplified in a human salivary gland adenocarcinoma. Proc. Natl. Acad. Sci. USA **82**:6497–6501.
18. **Semba, K., Y. Yamanashi, M. Nishizawa, J. Sukegawa, M. Yoshida, M. Sasaki, T. Yamamoto, and K. Toyoshima.** 1985. Location of the c-*yes* gene on the human chromosome and its expression in various tissues. Science **227**:1038–1040.
19. **Schubert, D., S. Heinemann, W. Carlisle, H. Tarikas, B. Kimes, J. Patrick, J. H. Steinbach, W. Culp, and B. L. Brandt.** 1974. Clonal cell lines from the rat central nervous system. Nature (London) **249**:224–227.
20. **Sillar, R. S., and B. D. Young.** 1981. A new method for the preparation of metaphase chromosomes for flow analysis. J. Histochem. Cytochem. **29**:74–78.
21. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
22. **Taub, R., I. Kirsch, C. Morton, G. Lenoir, D. Swan, S. Tronick, S. Aaronson, and P. Leder.** 1982. Translocation of the c-*myc* gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc. Natl. Acad. Sci. USA **79**:7837–7841.
23. **Ullrich, A., L. Coussens, J. S. Hayflick, T. J. Dull, A. Gray, A. W. Tam, J. Lee, Y. Yarden, T. A. Libermann, J. Schlessinger, J. Downward, E. L. V. Mayes, N. Whittle, M. D. Waterfield, and P. H. Seeburg.** 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (London) **309**:418–425.
24. **Xu, Y., S. Ishii, A. J. L. Clark, M. Sullivan, R. K. Wilson, D. P. Ma, B. A. Roe, G. T. Merlino, and I. Pastan.** 1984. Human epidermal growth factor receptor cDNA is homologous to a variety of RNAs overproduced in A431 carcinoma cells. Nature (London) **309**:806–810.
25. **Yamamoto, T., H. Hihara, T. Nishida, S. Kawai, and K. Toyoshima.** 1983. A new avian erythroblastosis virus, AEV-H, carries *erb*B gene responsible for the induction of both erythroblastosis and sarcomas. Cell **34**:225–232.
26. **Yamamoto, T., T. Nishida, N. Miyajima, S. Kawai, T. Ooi, and K. Toyoshima.** 1983. The *erb*B gene of avian erythroblastosis virus is a member of the *src* gene family. Cell **35**:71–78.
27. **Yoshida, M. C., T. Ikeuchi, and M. Sasaki.** 1975. Differential staining of parental chromosomes in interspecific cell hybrids with a combined quinacrine and 33258 Hoechst technique. Proc. Jpn. Acad. **51**:184–187.
28. **Young, B. D., M. A. Ferguson-Smith, R. Sillar, and E. Boyd.** 1981. High-resolution analysis of human peripheral lymphocyte chromosomes by flow cytometry. Proc. Natl. Acad. Sci. USA **78**:7727–7731.
29. **Yunis, J. J.** 1981. New chromosome techniques in the study of human neoplasia. Hum. Pathol. **12**:540–549.