J.Cell.Mol.Med. Vol 6, No 2, 2002 pp. 151-159

Review

Chromosome-mediated alterations of the MYC gene in human cancer

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Received: April 30, 2002; Accepted: May 14, 2002

Abstract

The step-wise accumulation of genetic and epigenetic alterations in cancer development includes chromosome rearrangements and viral integration-mediated genetic alterations that frequently involve proto-oncogenes. Proto-oncogenes deregulation lead to unlimited, self-sufficient cell growth and ultimately generates invasive and destructive tumors. C-MYC gene, the cellular homologue of the avian myelocitic leukemia virus, is implicated in a large number of human solid tumors, leukemias and lymphomas as well as in a variety of animal neoplasias. Deregulated MYC expression is a common denominator in cancer. Chromosomal rearrangements and integration of oncogenic viruses frequently target MYC locus, causing structural or functional alterations of the gene. In this article, we illustrate how genomic rearrangements and viruses integration affect MYC locus in certain human lymphomas and solid tumors.

Keywords: Breast cancer - Burkitt's lymphoma - hepatocellular carcinoma - MYC oncogene - chromosome alterations - viral integration - gene activation

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Introduction

Cancer arises from a single precursor cell by cumulative acquisition of multiple genetic and epigenetic alterations caused by diverse types of damage triggered by various mutagens, oncogenic viruses, errors in replication or the effects of aging. Genetic alterations and changes in DNA methylation may affect a variety of genes; proto-oncogenes, tumor suppressor genes, DNA mismatch repair genes and genes associated with cell aging or apoptosis. DNA damage that can be manifested as point mutations, chromosomal rearrangements, amplifications and deletions accumulate resulting in the emergence of incipient clonal cancer cells that acquire unlimited, self-sufficient growth and resistance to normal homeostatic regulatory mechanisms. Step-wise accumulation of cell alterations is necessary for the initiation of transformation and the acquisition of an invasive phenotype, even in cases with a preexisting germ line mutation of a tumor suppressor gene [1, 2].

Chromosomal abnormalities have been implicated in cancer development since the turn of the last century. However, only during the last two decades, with advances in cytogenetics and molecular biology, has the genetic basis of neoplasia been firmly established, with chromosomal alterations being recognized as critical in the pathogenesis of human cancer. Recurrent chromosomal alterations provide cytological and molecular markers for the diagnosis and prognosis of disease. They also facilitate the identification of genes that are important in carcinogenesis and, ultimately, may lead to the development of targeted therapy. Chromosomal changes frequently involve proto-oncogenes and their deregulation is associated with the development of cancer [1, 3, 4].

Members of the MYC gene family are involved in regulation of growth and development of normal and cancer cells. In particular, c-MYC, the cellular homologue of the avian myelocitic leukemia virus, is implicated in over 80% of human cancers and in a variety of animal neoplasias [5]. The MYC protein plays a well defined role as a component of signal transduction pathways promoting both cell proliferation and apoptosis. Deregulated MYC expression is a common denominator in cancer [6]. In this article, we will highlight and present our observations on the role of chromosome changes and viral integration in deregulation of MYC expression in several forms of cancer.

Chromosome translocations - mediated alterations of the MYC gene in lymphoma

A large number of recurrent and specific structural alterations have been identified in leukemias, lymphomas, and solid tumors [3, 4]. Leukemias, lymphomas and sarcomas commonly elicit specific balanced translocations that mediate activation of proto-oncogenes by their juxtaposition with promoter sequences or generate fusion of genes. As both oncogene products and gene fusion proteins are often transcriptional factors, the disruption of transcription control might be a critical and etiologically relevant alteration in these forms of cancer. The best known example is balanced translocation 9;22 in chronic myelocytic leukemia (CML) that is intimately associated with the initiation of the disease, provides a reliable marker for diagnosis and detection of residual disease and lead to the development of gleevek (SU5416), a ABL/BCR tyrosine kinase inhibitor, which is an effective therapeutic agent in CML and possibly in other forms of cancer [7]. The development of this therapeutic agent illustrates how, in time, progress in basic research leads to clinical advances. Philadelphia chromosome, a characteristic alteration in CML, was discovered over forty years ago and later proved to be an equal exchange between chromosomes 9 and 22, harboring at the site of reunion ABL and BCR genes which, as the result of chromosome reorganization, fused and formed ABL/BCR chimeric gene coding a protein with oncogenic properties.

Characteristic for Burkitt's lymphoma (BL) are translocations involving the MYC locus on chromosome 8q24 to any of the three immunoglobulin genes loci on chromosomes 14q32, 2p11 or 22q11[8]. The breakpoints in most common translocations t(8;14) cluster within or near MYC locus [9]. Due to the influence of immunoglobulin enhancer sequences placed near its locus, MYC gene exhibits

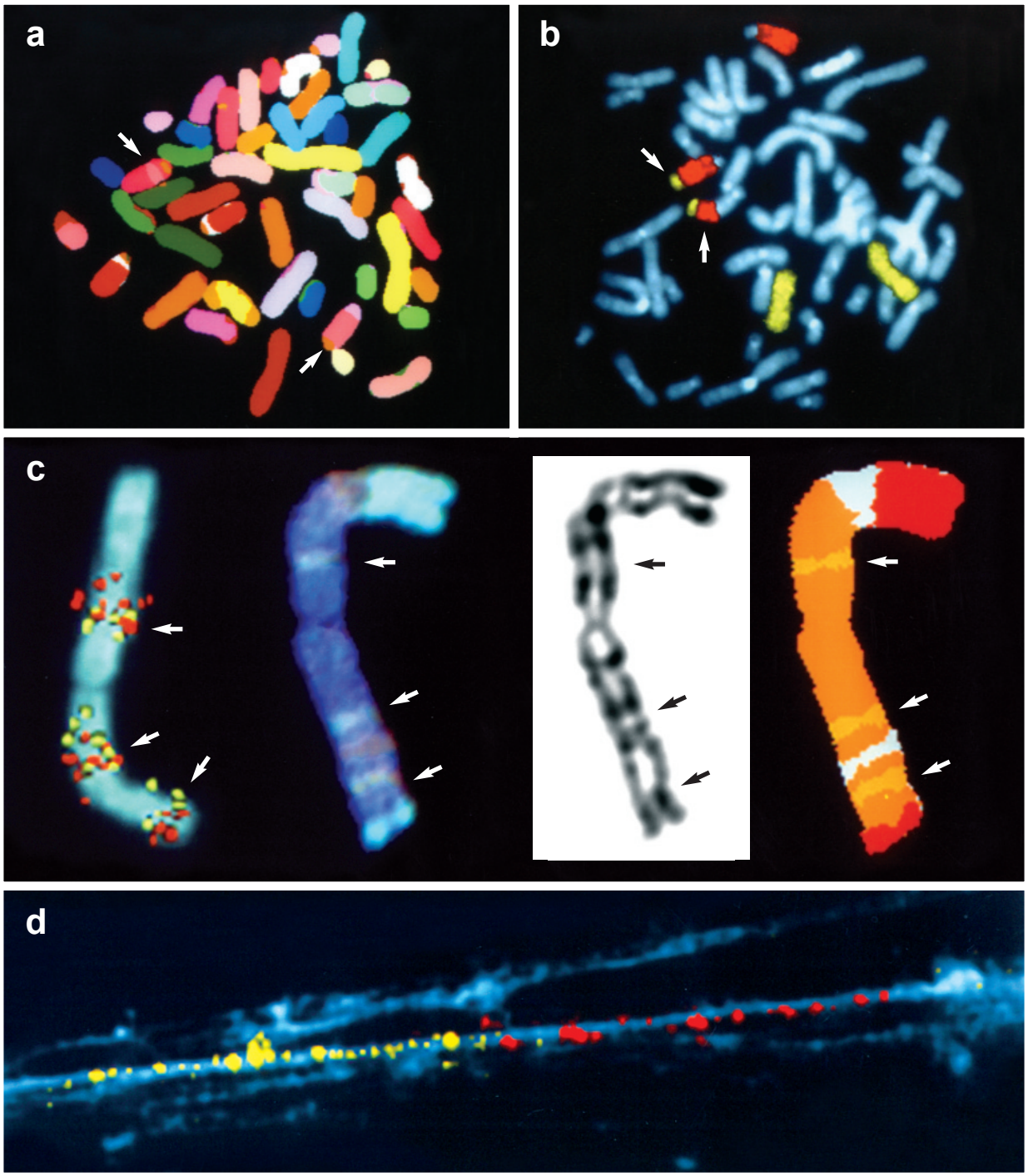

Fig. 1 Molecular cytogenetic characterization of a Burkitt's lymphoma cell line and the derivation of an complex abnormal chromosome from a breast carcinoma cell line. **a)** Classified SKY metaphase from Burkitt's lymphoma ST486 cell line carrying a novel translocation t(8;14;18) in addition to t(8;14). **b)** The derivation of this translocation is confirmed by painting with chromosomes 8 (yellow) and 14 specific probes (red). In both panels arrows indicate the abnormal chromosomes. **c)** Coamplification of ERBB2 and MYC oncogenes in an abnormal chromosome from breast cancer cell line. Shown from left to right are: clusters of multiple copies of ERB2 and MYC genes as depicted by two-color FISH, the pattern of SKY hybridization indicating the multi-chromosomal origin of the derivative chromosome, the G-banding pattern showing the presence of abnormally banded regions, and the classified SKY image showing that the abnormal derivative contains material from chromosomes 8, 3, 17 and 13. Arrows indicate that the sites of oncogenes clusters correspond to abnormally banded regions containing material from chromosome 17. **d)** FISH with MYC and ERBB2 probes on extended chromatin fibers showing proximity of two sets of signals.

abnormal expression [10, 11]. The translocations occur at an early stage of B-cell ontogeny as a result of V-D-J recombinase malfunction, and MYC gene alterations mediated by translocations have etiological significance to the development of BL [12, 13].

In two BL cell lines, CA46 and ST486, that have been used to clone and sequence the translocated MYC gene, the breakpoints involve an almost identical site within the first intron of the MYC gene [14]. In both lines, the first noncoding exon of the gene is retained on chromosome 8, while the coding sequences of the second and third exon are translocated to and rearranged with different regions of the IgH locus on chromosome 14**.** We analyzed these lines by spectral karyotyping (SKY), G-banding, fluorescence in situ hybridization (FISH) with chromosome painiting and single-copy gene probes, and comparative genomic hybridization (CGH). In addition to t(8; 14) translocation involving the MYC and IgH loci, we identified two new complex rearrangements, a t(7; 8; 14) translocation in CA 46 cells and t(8; 14; 18) in ST486 cells. In Fig. 1 a and b, the derivation of t(8;14;18) translocation as depicted by SKY and confirmed by chromosome painting with probes for chromosome 8 (yellow) and 14 (red) is illustartred. Both secondary translocations resulted in transposition of MYC sequences in a new genomic configuration [15]. When tumorigenicity of these lines was tested in SCID mice, CA46 cells produced tumors two weeks after cell inoculation while ST486 cells induced only one tumor after a long latency period. CGH and FISH analysis with specific YAC probes revealed partial duplication of the long arm of chromosome 1 which is the second most common alteration in BL and is known to be associated with aggressive tumors and poor prognosis [16]. Duplication of the bands 1q23-24 commonly observed in Epstein-Barr virus (EBV)-negative lines was identified only in highly tumorigenic CA46 cells suggesting that this region harbors gene(s) associated with tumor cell invasiveness. These observations suggest that MYC gene deregulation is associated with the initiation of neoplasia while duplication of an unknown gene on the long arm of chromosome 1 may occur during cancer progression and confers an aggressive phenotype.

MYC gene amplification and overexpression in breast cancer

Comparative genome hybridization that allows positional identification of gains and losses of DNA sequences in the entire tumor genome is based on competitive FISH of differentially labeled total genomic DNA from appropriate control tissue and tumor DNA to normal chromosome spreads. CGH has been applied to identify the gains and losses of DNA sequences in breast tumors, tumor-derived cell lines, and archival tumor material. CGH data obtained from 698 primary tumors and 38 established tumor cell lines have recently been compiled [17]. Despite large numbers of complex genomic changes in breast cancer, CGH screening showed that gain of the long arm of chromosomes 1 and 8 and loss of 13 are represented in over 90% of the cases [18]. Regions of gain frequently affect the distal part of the long arm of chromosome 8 containing MYC gene. Furthermore, a recent CGH ratio measurement has revealed that gain of 8q24 correlates with poor patient survival and is associated with mutation of the P53 tumor suppressor gene. The association between gain at 8q24 and p53 mutation might be attributable to transactivation of the MYC gene by the p53 promoter [19].

The amplification of MYC in primary breast tumors and tumor cell lines is also associated with the formation of a distinct type of chromosome changes referred as double minute chromosomes and abnormally banded or homogeneously stained regions (HSR). Both abnormalities reflect DNA amplification and like other structural alterations are associated with genomic instability and contribute to the process of carcinogenesis [1, 20-22]. Their occurrence leads to loss of control of copy gene number that may generate subpopulations of tumor cells with increased growth potential, invasiveness and refractory to chemotherapeutic agents [20-22]. These alterations are also viewed as an important in cancer development because they frequently involve proto-oncogenes which can alter the proliferation of the cells [1]. High-level amplification MYC and ERBB2 was demonstrated in breast cancer cell lines by FISH with genomic probes. Clusters of multiple copies of both genes were observed at the

site abnormally banded regions and HSRs in highly rearranged chromosomes, some originating from several different chromosomes (Fig. 1c). Although on chromosome preparations ERBB-2 and MYC signals appeared closely spaced, FISH on DNA fibers showed that the clusters may contain additional amplified genes (Fig. 1d). Amplification and overexpression of ERBB2 gene is a molecular and cytological marker for disease recurrence and led to the development of an anti-ERBB2 therapeutic agent, trastuzumab (herceptin), for breast cancer [23-25]. Tumor cell lines containing amplification of both MYC and ERBB2 as well as having a high incidence of genomic abnormalities were the most aggressive in nude mice [26].

MYC gene amplification in breast tumorigenic cells generated *in vitro*

A major advance in the understanding of the process of cell immortality and the acquisition of malignant transformation came from the successful and reproducible approach to generating in vitro tumorigenic transformants by sequentially inserting a genomic version of the SV40 large-T (LT) oncogene, the hTERT, the telomerase catalytic subunit and an oncogenic allele of the H-RAS gene into normal human kidney embryo cells and foreskin fibroblasts [27]. This strategy was extended to transform normal mammary cells and thus, for the first time epithelial cells from reduction mammoplasty have been converted into invasive, malignant cells by introducing a defined set of genes. The cytogenetic makeup of fibroblasts, kidney epithelial and mammary tumorigenic cells was examined by SKY. Fibroblast tumorigenic cell lines were highly aneuploid, kidney lines had a large fraction of karyotipically normal cells and mammary tumorigenic cell lines exhibited nonrandom alterations resulting in amplification of the MYC gene [28, 29]. Translocation and partial duplication, trisomy and isochromosome formation involving chromosome 8 were responsible for MYC gene amplification and concomital elevated MYC expression which occurred in cells derived from different tissue samples, in low and high RAS expressing populations and prior to cells injection into nude mice. Missdisjunction during cell division, chromosome translocations and formation of isochromosomes accounted for changes in MYC gene copy-number. These cytogenetic observations indicate that in addition to the inactivation of the p53 pathway and deregulation of retinoblastoma (pRB) chromosome and MYC gene alterations are required for tumorigenicity. MYC gene alterations may be an early event in breast cancer development. Noteworthy is evidence incriminating MYC gene in the development of benign breast lesions in normal breast tissue adjacent to breast carcinomas [30].

Oncogenic viruses integration at MYC gene locus

Several DNA and RNA viruses have been implicated as causative agents or cofactors in certain forms of human cancer. Retroviruses, the first filterable agents that cause cancer in chicken, are obligate mutagens since their replication cycle and persistence require integration into the host chromosomal DNA [31, 32]. Unlike retroviruses, the integration of certain DNA viruses into the chromosomal DNA was not considered a requirement for viral persistence. This view has been challenged by evidence for human papillomaviruses (HPV's) integration in most invasive genital cancers, hepatitis B virus (HBV) integration in the majority of hepatocellular carcinomas, and EBV persistence in integrated form on infected lymphoblastoid cells or BL. HPVs, avian, murine and feline leukemia viruses and woodchuck hepatitis virus (WHV) are known to integrate near MYC locus leading to gene activation [33].

Integration of the viral genome into cellular DNA can trigger a variety of interactions with their host cells that may be relevant for the induction of cell transformation, the maintenance of the transformed phenotype and tumor progression [34-35]. Viral integration leads to the acquisition by the host genome of viral transforming genes with new regulatory elements involving either the loss of viral regulatory mechanism or the addition of others of cellular origin. In the latter case, alterations of the cellular genes may result from the integrated viral sequences either as a consequence of the viral insertion per se or by the effect of viral regulatory elements on nearby cellular genes.

The specificity of viral integration is fundamental in determining the biological significance of the phenomenon to various pathological conditions including cancer [33-35]. The concept of nonrandom viral integration at fragile sites (FS) and regions of growth regulatory genes introduced nearly ten years after the first demonstration of HPV integration at FS in HeLa cells and the realization that most, if not all, oncogenic viruses preferentially integrate at FS and loci of genes involved in cell growth regulation [36, 37]. FS are regions susceptible to breakage under specific experimental conditions. Antifolates, fluorodeoxyuridin, methotrexate and aphidicolin, a DNA polymerase inhibitor induce the expression of FS during metaphase as nonstaining gaps or breaks usually involving both chromatids. Although FS are dispersed throughout the genome they tend to cluster at G-light chromosome bands that are known to contain GC-rich Alu repeats and are constitutionally more relaxed and unfolded during transcription. FS also correspond to nuclease sensitivity sites and zinc finger protein domains, indicating that FS reflect an aberrant state of genetic activity at areas associated with transcriptional regulation. FS tend to replicate late during cell cycle thus being limited available targets for breakage and recombination (38). Viral transforming genes E6, E7, and E1 and the regulatory region of HPV18 located near MYC genes were amplified and MYC constitutively expressed, possibly contributing to the acquisition of immortality and proliferative capacity in HeLa cells [39]. Subsequently, others and we provided compelling cytogenetic and molecular evidence for HPV and other viruses integration at preferred sites of fragility and regions of growth regulatory genes [40, 41]. Forty per cent of the HPV integration sites localized in cervical carcinomas cluster at FS 8q24 and the locus of MYC gene [42]. Invariably, HPV integration at 8q24 is accompanied by amplification of the viral sequences and MYC overexpression, which is considered to be a prognostic marker for invasiveness and metastatic potential in cervical cancer [39, 43].

Unexpected observations have been made with recombinant adeno–associated virus (AAV) in HeLa cells. The introduction of genes to correct hereditary defects or to suppress cancer is an emerging area of research. Retroviruses or genetically engineered vectors containing subgenomic viral domains need to be analyzed for delivery efficiency and possible adverse effects. Compelling evidence for chromosomal site-specific integration has been provided for AAV genome on a specific region of the chromosome 19. AAV high specificity for site integration has implications not only in understanding molecular mechanisms of viral integration but also for targeted gene transfer in human gene therapy [44, 45].

In contrast to the wild type virus, recombinant AVV did not integrate in chromosome 19 as demonstrated by Southern blot analysis and FISH of selected HeLa clones. However, integration did not appear to be random; 3 of the 5 unique clones studied had similar integration sites in areas close to known proto-oncogenes and FS which included integrations near MYC locus. These observations are relevant to the understanding of the mechanism involved in both the site–specific integration of the wild type AAV as well as this nonrandom integration of recombinant AAV that should allow safer approaches for genetic manipulation of cells [46].

MYC gene alterations in liver cancer

WHV, which has a genomic structure similar to that of HBV, which is closely associated with the development of HCC, exhibits striking similarities to retroviruses in terms of integration and requirements of RNA intermediate and reverse transcriptase for replication. WHV is most oncogenic of the hepandaviruses and is also known to integrate near MYC locus and cause gene activation. WHV-mediated rearrangements and activation of MYC mirror those resulting from chromosome translocations in hematological malignancies [47, 48].

This may also be the case in HCC developed in patients chronically infected with HBV. However, due to the inherent capacity of HBV to cause secondary chromosomal rearrangements, the localization of HBV integration sites in HCC may not represent the initial site of integration [49]. Independent of HBV integration near MYC locus, gains at 8q24 are recurrent in both HCC cell lines and primary tumors and involves MYC gene [49]. The importance of MYC gene in hepatocarcinogenesis has been firmly demonstrated both in human

tumors and in a transgenic mice model. Coexpession of MYC and transforming growth factor (TGF) alpha enhances the development of HCC in transgenic mice, through the disruption of the pRb/EF2 pathway. In addition, TGF alpha may function as a survival factor for neoplastic cells and thereby accelerates the neoplastic process [50-53]. Gain at 8q24 and MYC gene amplification in human HCC is frequently due to isochromosome formations and complex rearrangements of 8q [54].

Substantial progress has been achieved both in the identification of genes located at sites of recurrent chromosomal alterations and in profiling gene expression through the application of powerful cytogenetic and functional genomic techniques. Characterization of the molecular pathology and gene expression profiles of various cancers should provide both new clinical tools for the accurate diagnosis and prediction of prognosis as well as new targets for the development of therapeutic agents [55]. The MYC signaling network is indeed a very attractive molecular target in a variety of cancers.

References

1. **Bishop J.M.,** The molecular genetics of cancer, *Science*, **235:** 305-311, 1987
2. **Hanahan D., Weinberg, R.A.,** The hallmarks of cancer, *Cell*, **100:** 57-70, 2000
3. **Rabbitts T.H.,** Chromosomal translocations in human cancer, *Nature,* **372:** 143-149, 1994
4. **Mitelman F., Mertens F., Johansson B.,** A breakpoint map of recurrent chromosomal rearrangements in human neoplasia, *Nat. Genet.,* **15:** 417-474, 1997
5. **Grisham J.W.,** Interspecies comparison of liver carcinogenesis implications for cancer risk assessment, *Carcinogenesis*, **18:** 59-81, 1996
6. **Hueber A.O., Evan G.I.,** Traps to catch unwary oncogenes, *Trends Genet.,* 14: 364-367, 1998
7. **O'Dwyer M.E., Mauro M.J., and Druker B.J.,** Recent advancement in the treatment of chronic myelogenous leukemia, *Annu. Rev. Med.,* **53:** 369-381, 2002
8. **Nowell P., Dalla-Favera R., Finan J., Erikson J., Croce C.,** Chromosome translocations, immunoglobulin genes, and neoplasia. In Rowley J.D., Ultmann J. E. (eds). Chromosomes and cancer: from molecules to man. New York: Academic Press; 1983, pp. 165-181
9. **Dalla-Favera R., Bregni M., Erikson J., Patterson D., Gallo R.C., Croce C.M.,** Human c-myc oncogene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells, *Proc. Natl. Acad. Sci. U .S. A.,* **79:** 7824-7827, 1982
10. **Pelicci P.G., Knowles D.M.D., Magrath I., Dalla-Favera R.,** Chromosomal breakpoints and structural alterations of the c-myc locus differ in endemic and sporadic forms of Burkitt lymphoma, *Proc. Natl. Acad. Sci. U. S. A.,* **83:** 2984-2988, 1986
11. **Croce C.M., Nowell P.C.,** Molecular basis of B-cell neoplasia, *Blood,* **55:** 1-7, 1985
12. **Croce C.M.,** Role of chromosome translocation in human neoplasia, *Cell,* **49:** 155-156, 1987
13. **Haluska F.G., Tsujimoto Y., Russo G., Isobe M., Croce C.M.,** Molecular genetics of lymphoid tumorigenesis, *Prog. Nucleic Acid Res. Mol. Biol.,* **36:** 269-280, 1989
14. **Showe L.C., Ballantine M., Nishikura K., Erikson J., Kaji H., Croce C.M.,** Cloning and sequencing of a c-myc oncogene in a Burkitt's lymphoma cell line that is translocated to a germ line alpha switch region, *Mol. Cell. Biol.,* **5:** 501-509, 1985
15. **Zimonjic D.B., Keck-Waggoner C.L., Popescu N. C.,** Novel genomic imbalances and chromosome translocations involving c-myc gene in Burkitt's lymphoma, *Leukemia,* **15:** 1582-1588, 2001
16. **Gurtsevitch V.E., O'Conor G.T., Lenoir G.M.,** Burkitt's lymphoma cell lines reveal different degrees of tumorigenicity in nude mice, *Int. J. Cancer.,* **41:** 87-95, 1988
17. **Forozan F., Mahlamaki E. H., Monni O., Chen Y., Veldman R., Jiang Y., Gooden G. C., Ethier S. P., Kallioniemi A., Kallioniemi O.P.,** Comparative genomic hybridization analysis of 38 breast cancer cell lines: a basis for interpreting complementary DNA microarray data, *Cancer Res.,* **60:** 4519-4525, 2000
18. **Tirkkonen M.T.M., Karhu R., Kallioniemi A., Isola J., Kallioniemi O.P.,** Molecular cytogenetics of primary breast cancer by CGH, *Genes Chromosomes Cancer*, **21:** 177-184, 1998
19. **Jain A. N., Chin K., Borresen-Dale A-L, Erikstein B.K., Lonning P.E., Kaaresen R., Gray J.W.,** Quantitative analysis of chromosomal CGH in human breast tumors associates copy number abnormalities with p53 status and patient survival, *Proc. Natl. Acad. Sci. U. S. A.,* **98:** 7952-7957, 2001
20. **Alitalo K., Schwab M.,** Oncogene amplification in tumor cells. *Adv. Cancer Res.,* **47:** 235-281, 1986
21. **Schimke R.T.,** Gene amplification in cultured cells. *J. Biol. Chem.,* **63:** 5989-5992, 1988
22. **Pardue M.L.,** Dynamic instability of chromosomes and genomes, *Cell,* **66:** 427-431, 1991

23. **Kraus M.H., Popescu N.C., Amsbaugh S.C., King C.R.,** Overexpression of the Egf receptor-related proto-oncogene Erbb-2 in human mammary tumor cell lines by different molecular mechanisms, *EMBO J.,* **6:** 605-610, 1987
24. **Slamon D.J., Clark G.M., Wong S.G., Levin W.J., Ullrich A., McGuire W.L.,** Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene, *Science*, **235:** 177-182, 1987
25. **Pegram M.D., Lipton A., Hayes D.F., Weber B. L., Baselga J.M., Tripathy D., Baly D., Baughman S.A., Twaddell T., Glaspy J.A., Slamon D.J.,** Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185 HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment, *J. Clin. Oncol.,* **16:** 2659-2671, 1998
26. **Zimonjic D.B., Keck-Waggoner C.L., Yuan B.Z., Kraus M.H., Popescu N.C.,** Profile of genetic alterations and tumoigenicity of human breast cancer cells, *Int. J. Oncol.,* **16:** 221-230, 2000
27. **Hahn W.C., Counter C.M., Lundberg A.S., Beijersbergen R.L., Brooks M.W., Weinberg R. A.,** Creation of human tumor cells with defined genetic elements, *Nature*, **400:** 464-468, 1999
28. **Zimonjic D.B., Brooks M.W., Popescu N.C., Weinberg R.A., Hahn W.C.,** Derivation of human tumor cells *in vitro* without widespread genomic instability, *Cancer Res.,* **61:** 8838-8844, 2001
29. **Elenbaas B., Spirio L., Koerner F., Fleming M. D., Zimonjic D.B., Donaher J. L., Popescu N. C., Hahn, W. C., Weinberg R.A.,** Human breast cancer cells generated by oncogenic transformation of primary mammary epithelial cells, *Genes Dev.,* **15:** 50-65, 2001
30. **Lundin C., Mertens F.,** Cytogenetics of benign breast lesions, *Breast Cancer Res. Treat.,* **51:** 1-15, 1998
31. **Varmus H.,** Retroviruses, *Science,* **240:** 1427-1435, 1988
32. **Peters G.,** Oncogenes at viral integration sites, *Cell Growth Diff.,* **1:** 503-510, 1990
33. **Popescu N.C.,** Chromosome fragility and instability in human cancer, *Crit. Rev. Oncog.,* **5:** 121-140, 1994
34. **Weinberg R.A.,** Integrated genomes of animal viruses, *Ann. Rev. Biochem.,* **49:** 197-226, 1980
35. **Croce C.M.,** Integration of oncogenic viruses in mammalian cells, *Int. Rev. Cytol.,* **71:** 1-17, 1981
36. **Popescu N.C., DiPaolo J.A., Amsbaugh S.C.,** Integration sites of human papilloma-virus 18 DNA sequences on HeLa cell chromosomes, *Cytogenet. Cell Genet.,* **44:** 58-62, 1987
37. **Popescu N.C., Zimonjic D.B, DiPaolo J.A.,** Viral integration, fragile sites and protooncogenes in human neoplasia, *Hum. Genet.,* **84:** 383-386, 1989
38. **Zimonjic D.B., Druck T., Ohta M., Kastury K., Croce C.M., Popescu N.C., Huebner K.,** Positions of chromosome 3p14. 2 fragile sites (FRA3B) within the FHIT gene, *Cancer Res.,* **57:** 1166-1170, 1997
39. **Lazo P., DiPaolo J.A., Popescu N.C.,** Amplification of the integrated viral transforming genes of human papillomavirus 18 and 5' flanking cellular sequence located near the myc proto-oncogene in HeLa cells, *Cancer Res.,* **49:** 4305-4310, 1989
40. **Wilke C.M., Hall B.K., Hoge A., Paradee W., Smith D.I., Glover, T.W.,** FRA3B extends over a broad region and contains a spontaneous HPV16 integration site: direct evidence for the coincidence of viral integration sites and fragile sites, *Hum. Mol. Genet.,* **5:** 187-195, 1996
41. **Thorland E.C., Myers S.L., Persing D.H., Sarkar G., McGovern R.M., Gostout, B.S., Smith, D.I.,** Human papillomavirus type 16 integrations in cervical tumors frequently occur in common fragile sites, *Cancer Res.,* **60:** 5916-5921, 2000
42. **Popescu N.C., DiPaolo J.A.,** Cytogenetics of cervical neoplasia, *Cancer Genet. Cytogenet.,* **60:** 214-215, 1992
43. **Riou G., Le M.C., Favre M., Jeannel D., Bowrhis J., Orth G.,** Human papilloma-virus-negative status and c-myc gene overexpression: independent prognostic indicators of distant metastasis for early-stage invasive cervical cancer, *J. Natl. Canc. Inst.,* 84: 1525-1526, 1992
44. **Kotin R.M., Menninger J.C., Ward D.C., Berns K.I.,** Mapping and direct visualization of a region-specific viral DNA integration site on chromosome 19q13-qter, *Genomics,* **10:** 831-834, 1991
45. **Samulski R.J., Zhu X., Xiao X., Brook J.D., Housman D.E., Epstein N., Hunter L.A.,** Targeted integration of adeno-associated virus (AAV) into human chromosome 19, *EMBO J.,* **10:** 3941-3950, 1991
46. **Rivadeneira E.D., Popescu N.C., Zimonjic D.B., Cheng G.S., Nelson P.J., Ross M.D., DiPaolo J. A., Klotman M.E.,** Sites of recombinant adeno-associated virus integration, *Int. J. Oncol.,* **12:** 805-810, 1998
47. **Moroy T., Marchio A., Etiemble J., Trepo C., Tiollais P., Buendia M.A.,** Rearrangement and enhanced expression of c-myc in hepatocellular carcinoma of hepatitis virus infected woodchucks, *Nature,* **324:** 276-279, 1986

48. **MizunoY., Murukami S., Matsushita F., Unoura M., Kobayasi K., Migita S., Hatori N., Ohno S.,** Chromosomal assignment of woodchuck hepatitis virus (WHV) DNA integration sites in a woodchuck hepatocellular carcinoma-derived cell line (WH257GE10), *Int. J. Cancer,* **43:** 652-657, 1989
49. **Zimonjic D.B., Keck C.L., Thorgeirsson S.S., Popescu N.C.,** Novel recurrent genetic imbalances in human hepatocellular carcinoma cell lines identified by comparative genomic hybridization. *Hepatology,* **29:** 1208-1214, 1999
50. **Murakami H., Sanderson N., Nagy P., Marino P., Merlino G.T., Thorgeirsson S. S.,** Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis: interaction of c-myc and TGF-α in hepatic oncogenesis, *Cancer Res.,* **53:** 1719-1723, 1993
51. **Santoni-Rugiu E, Nagy P., Jensen M.R., Factor V. M., Thorgeirsson S.S.,** Evolution of neoplastic development in the liver of transgenic mice co-expressing c-myc and transforming growth factor, *Am. J. Pathol.,* **149:** 407-428, 1996
52. **Santoni-Rugiu E., Jensen M.R., Thorgeirsson S. S.,** Disruption of the pRb/E2F pathway and inhibition of apoptosis are major oncogenic events in liver constitutively expressing c-myc and transforming growth factor alpha, *Cancer Res.,* **58:**123-134, 1998
53. **Sargent L.M., Zhou X., Keck C.L., Sanderson N. D., Zimonjic D.B., Popescu N.C., Thorgeirsson, S.S.,** Nonrandom cytogenetic alterations in hepatocellular carcinoma from transgenic mice overexpressing c-myc and transforming growth factor alpha in the liver, *Am. J. Path.,* **154:** 1047-1055, 1999
54. **Keck C.L., Zimonjic D.B., Yuan, B.Z., Thorgeirsson S.S., Popescu N.C.,** Nonrandom breakpoints of unbalanced chromosome translocations in human hepatocellular carcinoma cell lines, *Cancer Genet. Cytogenet.,* **111:** 37-44, 1999
55. **Popescu N. C., Zimonjic D.B.,** Chromosome and gene alterations in breast cancer as markers for diagnosis and prognosis as well as targets for therapy, *Am. J. Med. Genet.,* 2002 (In Press)