Published in final edited form as:

Cancer Genet Cytogenet. 2009 May ; 191(1): 17–26. doi:10.1016/j.cancergencyto.2008.12.014.

Recurrent and Non-Random DNA Copy Number and Chromosome Alterations in c-Myc Transgenic Mouse Model for Hepatocellular Carcinogenesis: Implications for Human Disease

Drazen B. Zimonjic, Veronika Ullmannova-Benson, Valentina M. Factor, Snorri S. Thorgeirsson, and Nicholas C. Popescu

Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD. 20892

Abstract

Mouse models for hepatocellular carcinoma (HCC) provide an experimental ground to dissect the genetic and biological complexities of human liver cancer and enhance our ability to gain insights into the relevance of candidate cancer genes. We examined, using spectral karyotyping (SKY) and array-based CGH (aCGH), seven cell lines derived from HCC spontaneously developed in transgenic c-Myc animals (c-Myc), and four cell lines established from tumors induced in nude mice by inoculation with the original c-Myc cells (nuMyc). All of those cell lines exhibited gain of material from chromosomes 5, 6, 8, 10, 11, 15 and 19, and DNA copy-number loss from chromosomes 2, 4, 7, 9, 12, 14, and X. In addition, several recurrent chromosome reorganizations were found including del(3), t(3;8), del(4) t(4;11), t(6;5), del(7), del(8), del(9), t(10;14), del(11) and del(16). Chromosome breakpoints underlying rearrangements clustered in the regions previously identified as important for the early stages of c-Myc induced hepatocarcinogenesis. The results strongly suggest the importance of recurrent breakage and loss of chromosomes 4, 9 and 14, and gain of chromosomes 15 and 19 in mouse liver neoplasia. Genomic changes observed in c-Myc HCC cell lines are also recurrent in HCC developed in a different other transgenic mouse models, in mouse spontaneous HCC and derivative cell lines, as well as in preneoplastic liver lesions induced with chemical carcinogens. Overall, our results demonstrate selective, non-random genomic changes involving chromosomal regions homologous to those implicated in human HCC.

INTRODUCTION

Cancer arises from a single precursor cell by cumulative acquisition of multiple genetic and epigenetic alterations. Genomic reorganizations are recognized as important in the pathogenesis of cancer, as a large number of recurrent and specific structural alterations resulting in oncogenic stimulation or in loss of tumor suppressive functions have been identified in leukemias, lymphomas, and solid tumors [1–3]. In the past years, there was much progress in the development of fluorescence in situ hybridizations (FISH)-based molecular cytogenetic methods, such as comparative genomic hybridization (CGH), spectral karyotyping (SKY) and high-resolution array-based CGH (aCGH), which allowed identification of several chromosomal regions of recurrent genomic imbalances in human hepatocellular carcinoma

Send correspondence to: Nicholas C. Popescu, Laboratory of Experimental carcinogenesis, national cancer Institute, 37 Convent Drive MSC 4262, Building 37, Room 4128B, Bethesda, MD 20892-4262, phone (301) 435-0296, e-mail E-mail: popescun@mail.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(HCC), involving genes implicated in regulatory pathways that control genomic integrity, cell proliferation and differentiation [4].

Increasing availability of animal models for various disorders point to their effectiveness as tool to identify and characterize critical pathogenic modifications relevant to human disease. In particular, mouse models of tumor suppressor genes and oncogenes are extremely useful to investigate various aspects of cancer genetics and preclinical therapeutic evaluation. Tumor-prone transgenic mice with uniform genetic background offer the distinct opportunity for identifying chromosome and gene alterations associated with early stages of neoplastic development and tumor progression.

In one such example, independent analyses of liver cancer development in WHV/c-Myc and c-Myc/TGF α transgenic mouse models [5–7] have clearly demonstrated the high oncogenic impact of the c-Myc gene activation. However, the examinations of the genomic changes in liver tumors from genetically engineered animals are scarce [8,9]). In this study, we combined SKY and aCGH to dissect genomic alterations in HCC cell lines (c-Myc cells) established from tumors spontaneously developed in transgenic mice overexpressing c-Myc oncogene controlled by albumin enhancer/promoter. In addition, to further analyze these cells and see if they elicit tumorigenicity, retain their karyotype, or if emerging new populations are selected under in vivo environment, we inoculated nude mice with cells from all c-Myc lines and from thus caused tumors derived several new cell lines (nuMyc). We found that explanted nuMyc cells, like original c-Myc cells, displayed similar non-random chromosome alterations, but also exhibited an increased DNA copy-number changes. Comparison of our data and previously published findings [10,11] revealed that a limited number of chromosomal changes are selected during liver tumor development

MATERIAL AND METHODS

Cell lines

Seven HCC cell lines (604D.02.T1.CL1, 604D02T1CL4, 604D02T2CL1, 620A03T2CL3, 614B02T1CL4, 648A01T1CL3 and 654A03T1) were isolated and cloned in our laboratory from spontaneous HCC developed in c-Myc transgenic mice described elsewhere [5,7]. In addition, four cell lines, named nuMyc-1, nuMyc-3, nuMyc-4 and nuMyc-6, were derived from tumors developed in athymic nude mice after s.c. transplantation of the original HCC cell lines, 654A03T1, 604D02T1CL4, 604D02T2CL1 and 620A03T2CL3, respectively.

Spectral karyotyping analysis

Chromosome preparations obtained from exponentially growing cultures were used for SKY hybridization according to a standard protocol with minor modifications as previously described in detail [12]. Six to ten complete sets of karyotypes, i.e. spectral, classified and G-banded, were established and used for analysis of each line. Changes, both structural and numeric, observed in two or more karyotypes, were considered as recurrent for a given line. Numerical changes were determined based on ploidy level and representation in chromosome rearrangements. The composite karyotype of each cell line was established according to official cytogenetic nomenclature [13].

Array-based Comparative Genomic Hybridization

For aCGH, mouse genomic DNA was isolated with a QIAamp DNA Mini Kit according to manufacturer protocol (Qiagen, Valencia, CA). Test and reference (Promega, Madison, WI) DNAs were labeled with Cy3 or Cy5 fluorescent dyes (Pharmacia, Piscataway, NJ) according to BioPrime Array CGH Genomic Labeling protocol (Invitrogen, Carlsbad, CA) and cleaned using Microcon YM-30 filters (Millipore, Billerica, MA). Hybridization was carried out using

Mouse Genome CGH Microarray 44A from Agilent Technologies (Santa Clara, CA) according to CGH Procedures for Genomic DNA Analysis (Agilent Technologies). Slides were hybridized for 20 hours, washed, scanned with an Agilent microarray scanner, and data were analyzed using Feature Extraction® and CGH Analytics® software packages (Agilent Technologies). Dye-reversal experiments, with reciprocal labeling of the test and reference DNA, were performed for each experiment to ensure the test's reliability.

RESULTS

All seven cell lines isolated from HCC developed in c-Myc transgenic mice (c-Myc cells) were aneuploid with chromosome number ranging from hypodiploid (32) to hypertetraploid (87). Cell lines derived from tumors grown after inoculation of nude mice with original c-Myc cell lines (nuMyc cells) were also aneuploid but ranged from hyperdiploid (41) to hypersextaploid (137). Composite karyotypes (cp) of seven c-Myc and four nuMyc cell lines are presented in Tables 1 and 2, respectively.

The advantage of combining SKY and aCGH to examine the genomic alterations in these HCC cell lines is clearly illustrated in Figure 1. Generally, the comparison of the data obtained through SKY and aCGH analyses showed a remarkable concordance and enabled precise annotation of gains and/or losses, such as small DNA gains in proximal regions of chromosomes 1 and 11 in 604D.02.T2.CL1 (Fig. 1a, b), or increased DNA copy number in the distal region of chromosome 5 in 620A.03.T2.CL3 resulting from an increased number of derivatives of chromosome 6 bearing translocated chromosome 5 material (Fig. 1c, d).

Both the original (c-Myc) and secondary (NuMyc) cells exhibited aneuploidy and a variety of structural chromosomal rearrangements which, taken together, contributed to abnormalities in DNA copy numbers involving several chromosomes. Interestingly, very similar pattern of chromosomal gains and losses characterized both types of cell lines (Table 3); gain of material from chromosomes 5, 6, 8, 10, 11, 15 and 19, and DNA copy-number loss from chromosomes 2, 4, 7, 9, 12, 14, and X. Most frequent gains involved chromosomes 6, 10, 11, 15 and 19; in one cell line we identified 6–20 double minutes (DM) originating from chromosome 19. Losses occurred repeatedly on chromosomes 4, 9, 14 and X.

In addition to DNA copy-number changes, we identified a number of structural chromosomal rearrangements (Fig. 2) such as del(3), t(3;8), del(4) t(4;11), t(6;5), del(7), del(8), del(9), t(10;14), del(11) and del(16). Reciprocal translocation t(5;6)(G2;G1) was invariably present in all cell lines although number of copies of individual derivative chromosomes 5 and 6 varied. Distal part of chromosome 5 in derivative chromosome 6 is the site of c-Myc transgene integration [10]. In addition, there were two major deletions such as frequent unbalanced recurrent translocations t(3;8)(F1;D1), t(4;11)(D2;B5) with variant t(4;11)(D2;B1B5), and t(10;14)(B1;E3). Deletions of chromosomes 4, 8, 9 and 11 frequently occurred at different breakpoints; breakpoints on chromosome 4 clustered in regions A, C and D (Fig. 3) whereas chromosomes 8, 9 and 11 broke frequently in regions B-C, A-B and A-B, respectively. The breakpoints in translocations involving chromosomes 4 and 11 occurred at some of the sites observed in those chromosomes' respective deletions, namely within the regions 4D2 and 11B4, respectively, whereas breakpoint at chromosome 14 was identified as 14E3.

To identify chromosome changes associated with tumor progression, nude mice were s.c. injected with the original c-Myc cells from all lines, and thus initiated tumors were used to isolate and establish secondary nuMyc cell lines. SKY and aCGH analysis of those cells revealed that a proportion of numerical and structural alterations observed in the original (c-Myc) cells remained preserved in the secondary (nuMyc) cells as well. Yet, secondary cells (Fig. 4b, d, f) showed an increased incidence of DNA copy-number changes compared to the

corresponding original c-Myc cell lines (Fig. 4a, c, e) and in some cases, almost a completely new pattern of gains and/or losses (Fig. 4a, b).

Not surprisingly, they also lost some of the preexisting, and exhibited a few novel structural rearrangements such as translocations t(1;8), t(2;9), t(4;5) and t(14;19), which were not detected in the original c-Myc cells. Two examples of this pattern of changes are presented in Table 4.

DISCUSSION

The molecular cytogenetic analysis of cell lines derived from HCC developed spontaneously in c-Myc transgenic mice, and from nude-mice tumors caused by HCC cells injection, identified chromosome changes nonrandomly involving specific chromosomes and chromosome sites which corresponded to the breakpoints described in early hepatic lesions [8]. Partial or complete gain of chromosomes 15 and 19 and loss of chromosomes 4, 9, and 14 were the most common alterations found in the c-Myc HCC cell lines. The same alterations were also demonstrated in HCC from WHV/c-Myc transgenic mice and HCC induced by chemical carcinogens by CGH and loss of heterozygosity (LOH) analyses [11,14,15]). More importantly, nonrandom and specific alterations detected in our and other studies involve chromosome regions containing cancer-related genes in human HCC. Together these findings suggest that a limited number of genomic alterations selected in the majority of tumors are likely to be critical in hepatocarcinogenesis.

The most common gain involves chromosome 15 that has large parts of homology with human chromosome 8q. Gain of 8q is highly recurrent in human HCC as well as in several other cancers. Invariably, the CGH pattern of chromosome 8 consists of the long arm gain and frequent amplification of the distal region where c-Myc gene is located [16]. Since both original (c-Myc) and secondary (nuMyc) cell lines express the c-Myc transgene, the increased copy number of chromosome 15 might enhance the effect of this powerful oncogene in the development of tumors. Gain at 8q24 and c-Myc gene amplification in human or murine cancer cells, including HCC is often due to the isochromosome formation, Robertsonian translocations or complex rearrangements of 8q [17–19]). Recently, a comprehensive study using a “mosaic” mouse model for HCC demonstrated that knockdown of tumor suppressor gene DLC1 cooperates with c-Myc to induce HCC [20].

Gain of chromosome 19 is an early recurrent alteration during chemically induced hepatocarcinogenesis in the mouse as well as in the primary HCC and preneoplastic liver lesions [14–15]. Chromosome 19 carries susceptibility genes for liver tumors and possibly oncogenes involved in hepatocarcinogenesis [21]. One of the examined c-Myc HCC cell lines contained 3–4 copies of normal chromosomes 19 and 5 to 25 double minute chromosomes (DM) derived from chromosome 19. DM and homogeneously stained regions are commonly associated with the acquisition of drug resistance and tumor progression and manifest DNA amplification [22,23]. We microdissected and generated PCR probe from DM and localized the sites of DNA amplification at regions 19B1.1-B1.3 and 19D1-D2 [9]. These regions harbor MXI1 and ms-Myc gene that interfere with c-Myc gene function, and RelA, a gene recognized to be important in regulation of malignant cell growth, survival and transformed phenotype [24–26]. We found that ms-Myc gene is located on chromosome X and not on chromosome 19 as was previously thought [27]

Deletion and LOH of chromosomes 4, 9 and 14 are highly recurrent events in HCC developed in both c-Myc and in WHV/c-Myc transgenic mice [11]. Deletions of chromosome 4 occur at different regions. The distal portion of chromosome 4 was the most frequently deleted region. A similar pattern was observed in mouse breast tumors [28]. Recurrent LOH of chromosome

4 affecting a region homologous to human chromosome 9 was detected in diethylnitrosoamine-induced-C3H/MSM mouse HCC [29]. Mouse chromosome 4 and human chromosome 1 suppresses the malignant phenotype in cell fusions of normal and tumor cells [30,31]). The mouse orthologues of at least three genes that are lost or mutated in several human cancers map to chromosome 4: the type I TGF- β receptor gene (TGFR1), the tightly linked genes for the p15INK4b (CDKN2B) and p16INK4a/p19ARF (CDKN2A) cyclin-dependent kinase inhibitors, and the p18INK4c cyclin-dependent kinase inhibitor gene (CDKN2C). The cyclin-dependent kinase inhibitors CDKN2/p16, p15 and p19 are deleted in murine HCC [32, also reviewed in 33].

Loss of chromosome 9B-ter was common in both original c-Myc cell lines and secondary nuMyc cells. Deletion of the same region has been observed in chemically induced liver tumors isolated from B6C3F1 mice [34]. The distal portion of mouse chromosome 9 is homologous to human 3p, a region with the highest fragility in human genome encompassing the FHIT gene [35]. The FHIT gene is expressed in normal human hepatic cells and is not expressed or is abnormally expressed in human and rodent primary HCC or HCC cell lines, suggesting the importance of the FHIT alterations in HCC development [36–38]. The transforming growth factor beta II receptor (TGF- β receptor II), mapped to mouse chromosome 9 and human 3p, is another candidate tumor susceptibility gene [39,40]). The expression of TGF- β II receptor was found to be downregulated in human HCC and in 80% of the c-Myc/TGF- β tumors [41,42]. Among bona fide and candidate tumor suppressor genes, on mouse chromosome 9 and human chromosome 3p, is also TMEM7 (transmembrane protein 7, RTP3) that shares substantial sequence homology with human and mouse 28-kDa interferon-alpha (IFN- α). Ectopic expression of TMEM7 in HCC lines inhibits HCC cell proliferation, colony formation, and cell migration *in vitro* and reduces tumor formation in nude mice. [43,44].

Deletion of chromosome 14D-ter was the second most common deletion in c-Myc tumor cell lines. In contrast, deletion 14 was not observed in the early lesions isolated from the c-Myc/TGF- α HCC [8], suggesting that, similar to other mouse malignancies [45], deletion 14 occurs at later stages of tumor development. Region of deletions 14D-ter is homologous to human chromosome regions 8p21-23 and 13q14. Both regions are recurrently underrepresented in HCC [16] and the region 8p21-23, in particular, harbors several candidate and bona fide tumor suppressor genes [46,47].

Significantly, near the reciprocal translocation t(5;6) with the breakpoint near the integration site of c-Myc transgene, which was present in all analyzed c-Myc and nuMyc cell lines, we identified a new gene [48]. Analysis of the genomic DNA flanking the c-Myc transgene revealed that the integration of the transgene had induced an approximately a 40-kb deletion that included the first exon of a new gene encoding GTF1IRD1 (general transcription factor II-I repeat domain containing protein 1). Human GTF2IRD1 is one of the 16 genes present in approximately 1.6 Mb interval commonly deleted in Williams-Beuren syndrome (WBS). Therefore, the c-Myc transgenic mouse carrying the t(5;6) translocation, represents the first “knockout” of one of the genes present in the WBS critical region. GTF2IRD1 encodes a widely expressed, multi-functional helix-loop-helix transcription factor, which binds to pRb, though its role in carcinogenesis remains elusive [48].

In conclusion, our results underline the value of mouse models of HCC for identification of non-random and recurrent genomic alterations that are homologous to human and harbor a limited set of genes relevant to hepatocarcinogenesis

Acknowledgments

This research was supported by the Intramural Research Program of the National Cancer Institute, NIH.

References

1. Sandberg, A. The chromosomes in human cancer and leukemia. Vol. 2. Elsevier Science Publishing; New York: 1990.
2. Mitelman F, Mertens F, Johansson BA. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat Genet* 1997;15:417–474. [PubMed: 9140409]
3. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
4. Thorgeirsson SS, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002;31:339–346. [PubMed: 12149612]
5. Murakami H, Sanderson N, Nagy P, Marino P, Merlino GT, Thorgeirsson SS. 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* 1993;53:1719–1723. [PubMed: 8467484]
6. Eteimbe J, Degott C, Renard CA, Fourel G, Shamoon B, Vitvitski-Trepo L, Hsu TY, Tiollais P, Babinet C, Buendia MA. Liver-specific expression and high oncogenic efficiency of a c-Myc transgene activated by woodchuck hepatitis virus insertion. *Oncogene* 1994;9:727–737. [PubMed: 8108115]
7. Santoni-Rugiu E, Nagy P, Jensen MR, Factor VM, Thorgeirsson SS. Evolution of neoplastic development in the liver of transgenic mice co-expressing c-Myc and transforming growth factor. *Am J Pathol* 1996;149:407–428. [PubMed: 8701981]
8. Sargent LM, Sanderson ND, Thorgeirsson SS. Ploidy and karyotypic alterations associated with early events in the development of hepatocarcinogenesis in transgenic mice harboring c-Myc and transforming growth factor α transgenes. *Cancer Res* 1996;56:2137–2142. [PubMed: 8616862]
9. Zimonjic DB, Zhang H, Shan Z, Factor VM, Trent J, Thorgeirsson SS, Popescu NC. DNA amplification associated with double minutes originating from chromosome 19 in mouse hepatocellular carcinoma. *Cytogenet Cell Genet* 2001;93:114–116. [PubMed: 11474192]
10. Sargent LM, Zhou X, Keck CL, Sanderson ND, Zimonjic DB, Popescu NC, Thorgeirsson SS. Nonrandom cytogenetic alterations in hepatocellular carcinoma from transgenic mice overexpressing c-Myc and transforming growth factor in the liver. *Am J Pathol* 1999;154:1047–1055. [PubMed: 10233843]
11. Wu Y, Renard CA, Apiou F, Huerre M, Tiollais P, Dutrillaux B, Buendia A. Recurrent allelic deletions at mouse chromosome 4 and 14 in Myc-induced liver tumors. *Oncogene* 2002;21:1518–1526. [PubMed: 11896580]
12. Zimonjic DB, Pollock J, Westerfield P, Popescu NC, Ley JT. Acquired, non-random chromosomal abnormalities associated with the development of acute promyelocytic leukemia in transgenic mice. *Proc Natl Acad Sci USA* 2000;97:13306–13311. [PubMed: 11087871]
13. Shaffer, LG.; Tommerup, N., editors. ISCN. An international system for human cytogenetic nomenclature. S. Karger; Basel: 2005.
14. Danielsen HE, Brogger A, Reith A. Specific gain of chromosome 19 in preneoplastic liver cells after diethylnitrosoamine treatment. *Carcinogenesis* 1991;12:1778–1780.
15. Ogawa K, Osanai M, Obata M, Ishizaki K, Kamiya K. Gain of chromosomes 15 and 19 is frequent in both mouse hepatocellular carcinoma cell lines and primary tumors, but loss of chromosome 4 and 12 is detected only in the cell lines. *Carcinogenesis* 1999;20:2083–2088. [PubMed: 10545409]
16. Zimonjic DB, Keck CL, Thorgeirsson SS, Popescu NC. Novel recurrent genetic imbalances in human hepatocellular carcinoma cell lines identified by comparative genomic hybridization. *Hepatology* 1999;29:1208–1214. [PubMed: 10094966]
17. Keck CL, Zimonjic DB, Yuan BZ, Thorgeirsson SS, Popescu NC. Nonrandom breakpoints of unbalanced chromosome translocations in human hepatocellular carcinoma cell lines. *Cancer Genet Cytogenet* 1999;111:37–44. [PubMed: 10326589]
18. Popescu NC, Zimonjic DB. Chromosome-mediated alterations of the myc gene in human cancer. *J Cell Mol Med* 2002;6:151–159. [PubMed: 12169201]
19. Guffei A, Lichtensztejn Z, Gonçalves dos Santos Silva A, Louis SF, Caporali A, Mai S. c-Myc-Dependent Formation of Robertsonian Translocation Chromosomes in Mouse Cells. *Neoplasia* 2007;9:578–588. [PubMed: 17710161]

20. Xue W, Krasnitz A, Lucito R, Sordella R, VanAelst L, Cordon-Cardo C, Singer S, Kuehnel F, Wigler M, Powers S, Zender L, Lowe SW. DLC1 is a chromosome 8p tumor suppressor whose loss promotes hepatocellular carcinoma. *Genes & Dev* 2008;22:1439–1444. [PubMed: 18519636]
21. Manenti G, Binelli G, Gariboldi M, Canzian F, Gregoria DL, Falvella FS, Dragani TA, Pierotti MA. Multiple loci affect predisposition to hepatocarcinogenesis in mice. *Genomics* 1994;23:118–124. [PubMed: 7829059]
22. Alitalo K, Schwab M. Oncogene amplification in tumor cells. *Adv Cancer Res* 1986;47:235. [PubMed: 3022564]
23. Schimke RT. Gene amplification in cultured cells. *J Biol Chem* 1988;263:5989–5992. [PubMed: 3283115]
24. Edelhoff S, Ayer DE, Zervos AS, Steingrimsson E, Jenkins NA, Copeland NG, Eisenman RN, Brent R, Distech CM. Mapping of two genes encoding members of a distinct subfamily of MAX interacting proteins: MAD to human chromosome 2 and mouse chromosome 6, and MXI1 to human chromosome 10 and mouse chromosome 19. *Oncogene* 1994;9:665–668. [PubMed: 8290278]
25. Sugiyama A, Noguchi K, Kitanaka C, Katou N, Tashiro F, Ono T, Yoshida MC, Kuchino Y. Molecular cloning and chromosomal mapping of mouse intronless myc gene acting as a potent apoptosis inducer. *Gene* 1999;226:273–283. [PubMed: 9931502]
26. Lemmer ER, Welch JL, Tsai T, Keck-Waggoner CL, Zimonjic DB, Huh C-G, Popescu NC, Thorgeirsson SS. Genomic structure and chromosomal localization of the mouse rela (p65) gene. *Cytogenet Cell Genet* 2000;89:129–132. [PubMed: 10894954]
27. Shan ZH, Popescu NC. Reassignment of Mycs gene to mouse chromosome XA1.2-2 by radiation hybrid mapping and fluorescence in situ hybridization. *Cytogenet Genome Res* 2002;97:152–153.
28. Hodgson JG, Malek T, Bornstein S, Hariono S, Ginzinger DG, Muller WJ, Gray JW. Copy number aberrations in mouse breast tumors reveal loci and genes important in tumorigenic receptor tyrosine kinase signaling. *Cancer Res* 2005;65:9695–9704. [PubMed: 16266989]
29. Miyasaka K, Ohtake K, Nomura K, Kanda H, Kominami R, Miyashita N, Kitagawa T. Frequent loss of heterozygosity on chromosome 4 in diethylnitrosamine-induced C3H/MSM mouse hepatocellular carcinomas in culture. *Mol Carcinog* 1995;13:37–43. [PubMed: 7766309]
30. Bieche I, Champeme MH, Matifas F, Cropp CS, Callahan R, Lidereau R. 2 Distinct regions involved in 1p deletion in human primary breast-cancer. *Cancer Research* 1993;53:1990–1994. [PubMed: 8097672]
31. Jonasson J, Povey S, Harris H. The analysis of malignancy by cell fusion. VII. Cytogenetic analysis of hybrids between malignant and diploid cells and of tumours derived from them. *J Cell Sci* 1977;24:217–254. [PubMed: 893544]
32. Drinkwater NR. Genetic control of hepatocarcinogenesis in C3H mice. *Drug Metab Rev* 1994;26:201–208. [PubMed: 8082565]
33. Grisham JW. Interspecies comparison of liver carcinogenesis implications for cancer risk assessment. *Carcinogenesis* 1996;18:59–81. [PubMed: 9054591]
34. Davies LM, Caspary WJ, Sakallah SE, Maronpot R, Wiseman R, Barrett JC, Elliott R, Hozier JC. Loss of heterozygosity in spontaneous and chemically induced tumors of the B6C3F1 mouse. *Carcinogenesis* 1994;15:1637–1645. [PubMed: 8055644]
35. Huebner K, Croce CM. FRA3B and other common fragile sites: the weakest links. *Nat Rev Cancer* 2001;1:214–221. [PubMed: 11902576]
36. Schlott T, Ahrens K, Ruschenburg I, Reimer S, Hartmann H, Droese M. Different gene expression of MDM2, GAGE-1,-2 and FHIT in hepatocellular carcinoma and focal nodular hyperplasia. *Br J Cancer* 1999;80:73–78. [PubMed: 10389981]
37. Yuan BZ, Keck-Waggoner CL, Zimonjic DB, Thorgeirsson SS, Popescu NC. Alterations of the FHIT gene in human hepatocellular carcinoma. *Cancer Res* 2000;60:1049–1053. [PubMed: 10706123]
38. Tsujiuchi T, Sasaki Y, Kubozoe T, Tsutmani M, Konishi Y, Nakae D. Alterations of the Fhit gene in hepatocellular carcinomas induced by N nitrosodiethylamine in rats. *Mol Carcinog* 2002;34:19–24.
39. Bonyadi M, Cui W, Nagase H, Akhurst RJ. The TGF beta type II receptor, Tgfb2, maps to distal mouse chromosome 9. *Genomics* 1996;33:328–329. [PubMed: 8660988]

40. Mathew S, Murty VV, Cheifetz S, George D, Massague J, Chaganti RR. Transforming growth factor receptor gene TGFB2 maps to human chromosome band 3p22. *Genomics* 1994;20:114, 115. [PubMed: 8020936]
41. Sue SR, Chari RS, Kong FM, Mills JJ, Fine RL, Jirtle RL, Meyers WC. Transforming growth factor-beta receptors and mannose 6-phosphate/insulin-like growth factor II receptor expression in human hepatocellular carcinoma. *Ann Surg* 1995;222:17–18.
42. Santoni-Rugiu E, Jensen MR, Thorgeirsson SS. 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* 1998;58:123–134. [PubMed: 9426068]
43. Imreh S, Klein G, Zabarovsky ER. Search for unknown tumor-antagonizing genes. *Genes Chromosomes Cancer* 2003;38:307–321. [PubMed: 14566849]
44. Zhou X, Popescu NC, Klein G, Imreh S. The interferon-alpha responsive gene TMEM7 suppresses cell proliferation and is downregulated in human hepatocellular carcinoma. *Cancer Genet Cytogenet* 2007;177:6–15. [PubMed: 17693185]
45. Pathak S, Dave BJ, Gadhia PK. Mouse chromosome 14 is altered in different metastatic murine neoplasias. *Cancer Genet Cytogenet* 1995;83:172–173. [PubMed: 7553591]
46. Birnbaum D, Adélaïde J, Popovici C, Charafe-Jauffret E, Mozziconacci MJ, Chaffanet M. *Lancet Oncol* 2003;4:639–42. [PubMed: 14554243]
47. Popescu NC. Fragile sites and cancer genes on the short arm of chromosome 8. *Lancet Oncology* 2004;5:77–77. [PubMed: 14761808]
48. Durkin ME, Keck-Waggoner CL, Popescu NC, Thorgeirsson SS. Integration of a c-Myc transgene results in disruption of the mouse Gtf2ird1 gene, the homologue of the human GTF2IRD1 gene hemizygously deleted in Williams-Beuren syndrome. *Genomics* 2001;73:20–27. [PubMed: 11352562]

Figure 1.
Spectral karyotypes (a, c) and aCGH profiles (b, d) in 604D.02.T2.CL1 and 620A.03.T2.CL3 cell lines, respectively

Figure 2.

Selected recurrent structural chromosome rearrangements identified in c-Myc and NuMyc cell lines. Combined use of SKY and aCGH enabled precise annotation and identification of even cryptic losses such as interstitial loss of material from chromosome 8 (a) in translocation (3;8), chromosome 11 (b) in complex reorganization involving chromosomes 4, 5 and 11, or chromosome 9 (c), or small proximal and distal deletions in chromosomes 10 and 14, respectively.

Figure 3.

Variable “shortening” of chromosome 4; deletions of this chromosome occur at different breakpoints, sometimes within the same cell line. Most frequent breakpoints are located in region D2 followed by C6 and A5.

Figure 4.

Cell lines isolated from tumors induced in nude mice by injection of the original c-Myc HCC cell lines show increased incidence of DNA copy-number changes. (a, b) 604D.02.T1.CL4 and nuMyc 3, (c, d) 604D.02.T2.CL1 and nuMyc 4, and (e, f) 620A.03.T2.CL3 and nuMyc

Table 1

Composite karyotypes of cells lines derived from HCC developed in c-Myc transgenic mice

1.	604D.02.T1.CL1 (CL1)	64–70, XXX, -X, del(3)(A3F1), der(3)t(3;8)(F1;D1), +del(4)(C6)[2] del(4)(D2)[4], der(4)t(4;11)(D2;B5)[4], del(5)(D)[5], der(5)t(5;6)(G2;G1)x3, der(6)t(5;6)(G2;G1)x3, del(7)(A2A3)[3], del(8)(A4B3)[5], del(9)(A5F1)[5], +11[5], -12[5], +der(14)t(10;14)(A4;E3), +15 [3], del(16)(C1)[4] +19[5] [cp6]
2.	604D.02.T1.CL4 (CL4):	64–71, XXX, -X, +del(2)(C1)[3], del(3)(A3F1)[6], der(3)t(3;8)(F1;D1), +del(4)(C6)[1], +del(4)(D2)[4], der(4)t(4;11)(D2;B5)[4], +5[3], der(5)t(5;6)(G2;G1)x3, der(6)t(5;6)(G2;G1)x3, del(7)(A2A3)[4], del(8)(A4B3)[3], del(9)(A5F1)[2], +11[4], -12[3], +der(14)t(10;14)(A4;E3), +15[5], del(16)(C1)[2], +19 [cp6]
3.	604D.02.T2.CL1 (T-2):	73–84, XXY, -Y[6], -Y[4], -X[2], -4[9], der(5)t(5;6)(G2;G1)x4, der(6)t(5;6)(G2;G1)x4, +del(6)(G1)[5], +8[2], del(9)(E1)[4], der(9)t(9;X)(E1;A1)[2], +11[2], -12[7], +14[2], +15[2], +17x2[2], +18[2], 1–10dm(19) [cp9]
4.	620A.03.T2.CL3 (T-20):	32–39, XX, -X, del(1)(A3)x2[3], del(4)(D2)[6], der(5)t(5;6)(G2;G1)x2, der(6)t(5;6)(G2;G1)x2, -7[2], +del(7)(A3)[2], del(7)(F1)[3], del(9)(A5), +del(11)(A3)x2[3], -13, -14, +16[7], +19[4] [cp9]
5.	614B.02.T1.CL4 (614)	69–75, XXXX, -X, -X, -1[6], -3, -4, del(4)(C6)[4], del(4)(D2)[5], der(5)t(5;6)(G2;G1)x4, der(6)t(5;6)(G2;G1)x4, +der(6)t(5;6)(G2;G1), -7, -9, +10[5], der(11)t(11;14)(A2;D3), -12, del(12)(C1), -14, del(14)(C3), +15, -16, +19 [cp9]
6.	648A.01.T1.CL3 (648):	61–69, XXX, -X, +der(1)t(1;17)(B;D), -2[6], del(4)(A5)[8], der(5)t(5;6)(G2;G1)x3, +der(5)t(5;6)(G2;G1)[6], der(6)t(5;6)(G2;G1)x3, +der(6)t(5;6)(G2;G1)[7], +7, +10[4], +11[7], -12, +15, +19 [cp9]
7.	654A.03.T1 (654)	43–46, XY, -X[1], -Y[3], del(4)(D1)[2], der(5)t(5;6)(G2;G1)x2, der(6)t(5;6)(G2;G1)2, +6[2], +10[2], +11[3], +13[3], +15 +19 [cp4]/77–84, XXY, -Y, -Y, del(4)(D1)x2[3], der(5)t(5;6)(G2;G1)x4, der(6)t(5;6)(G2;G1)x4, +del(6)(G1)[2], -8[2], +11[2], +13, +15, -16[2], +19 [cp3]

Table 2

Composite karyotypes of nuMyc cell lines derived from tumors developed in nude mice after s.c. transplantation of c-Myc HCC cell lines

8.	NuMyc-3 (mice injected with [2] 604D.02.T1.CL4)	56–62, XXX, -X, -X, del(2)(G)[8], del(3)(A3F1)[9], der(3)t(3;8)(F1;C1)[9], del(4)(D2), der(4)t(4;11)(D2;B1B5), -5[2], der(5)t(5;6)(G2;G1)x2, der(5) t(4;5)(D2;F), der(6)t(5;6)(G2;G1)x3, del(8)(C1), del(9)(B)[9], del(11)(B1B5), +del(12)(C3), -14, del(15)(D3), +16[3], del(16)(C3), +19, +19[3] [cp10]
9.	NuMyc-4 (mice injected with [3] 604D.02.T2.CL1)	81–137, XXXXXX, -X, -X, del(1)(B)[3], +3, -4, der(5)t(5;6)(G2;G1)x6, +der(5)t(5;6)(G2;G1)[6], der(6)t(5;6)(G2;G1)x6, +der(6)t(5;6)(G2;G1)[6], del(9)(B)[4], +11, +del(13)(A2)[3], del(14)(C1)x2[8], +15, der(19)t(14;19)(D3;C1)x2[6] [cp10]
10.	NuMyc-6 (mice injected with [4] 620A.03.T2.CL3)	54–66, XXX, -X, -X, +der(1)t(1;8)(C4;C2)x2[11], -2[8], -4, der(5)t(5;6)(G2;G1)x3, + der(5)t(5;6)(G2;G1)[5], +del(5)(B2)[7], der(6)t(5;6)(G2;G1)x4[10], +del(8)(A2B3)[6], del(9)(A5B)[6], der(9) t(2;9)(E4;E3)[11], -10[8], +del(11)(A)[6], +12[4], der(14)t(10;14)(A4;E3)x2[8], +15, der(16)t(16;X)(C2;?) [10], (+18[8], +19[4], +19[4] [cp9]
11.	NuMyc-1 (mice injected with [7] 654A.03.T1)	42–45, XX, -X, der(5)t(5;6)(G2;G1)x2, der(6)t(5;6)(G2;G1)x3, -9[2], +10, +11[5], +15[9], +19[9] [cp10]

Table 3

DNA copy-number gains and losses in c-Myc and nuMyc mouse cell lines

	GAIN	GAIN	LOSS	LOSS
Chr.	% c-Myc cell lines	% nuMyc cell lines	% c-Myc cell lines	% nuMyc cell lines
1	28			
2			28	50
3				
4	28		86	75
5	43	75		
6	86	75		
7			71	
8	43	50		
9			71	50
10	71	50		
11	86	75		
12		50	71	
13				
14			57	75
15	86	75		
16				50
17				
18				
19	100	100		
X			86	75
Y				

Changing pattern of structural rearrangements in two pairs of original (c-Myc) and secondary (nuMyc) cells lines that exhibited most of structural rearrangements. Printed in boldface are rearrangements lost or gained in the secondary cells

Table 4

Chr	604D.02.T1.CL4	NuMyc-3	620A.03.T2.CL3	NuMyc-6
1			del(1)	t(1;8)
2	del(2)	del(2)		
3	del(3) t(3;8)	del(3) t(3;8)		
4	del(4) t(4;11)	del(4) t(4;11)	del(4)	
5	t(5;6)	t(4;5) t(5;6)	t(5;6)	del(5) t(5;6)
6	t(6;5)	t(5;6)	t(5;6)	t(5;6)
7	del(7)		del(7)	
8	del(8)	del(8)		del(8)
9	del(9)	del(9)	del(9)	del(9) t(2;9)
11		del(11)	del(11)	del(11)
12		del(12)		
14	t(10;14)			t(10;14)
15		del(15)		
16	del(16)	del(16)		t(16;x)