Oncogene Research, 1987, Vol. 1, pp. 169–178
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Human *ros*1 and *mas*1 Oncogenes Located in Regions of Chromosome 6 Associated with Tumor-Specific Rearrangements

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Oncogenes have been implicated in tumorigenesis based on their localization to chromosomal sites associated with tumor-specific structural rearrangements. We have mapped the human *ros*1 (formerly *mcf*3) and *mas*1 oncogenes to the distal half of chromosome 6q, within a region frequently rearranged in malignant cells. Chromosomal mapping of these two new human transforming genes may help elucidate the involvement of the long arm of chromosome 6 in diverse tumor types.

KEYWORDS: *oncogenes, gene mapping,* in situ *hybridization*

INTRODUCTION

Localization of cellular oncogenes at sites associated with tumor-specific chromosomal rearrangements suggests these genes may function in the pathogenesis of some forms of human cancer. Burkitt's lymphoma cells are characterized by one of three chromosomal markers: a t(2;8), t(8;14), or t(8;22) in which the *myc* oncogene is activated by juxtaposition with one of the immunoglobin loci (Dalla-Favera *et al.*, 1982a; Taub *et al.*, 1982; de la Chappelle *et al.*, 1983; Croce *et al.*, 1983; Erikson *et al.*, 1983; Malcolm *et al.*, 1985; Hameister and Adolf, 1986). Chronic myelogenous leukemia is similarly characterized by the presence in malignant cells of a marker t(9;22), called the Philadelphia chromosome, in which the *abl* oncogene is activated by translocation into the breakpoint cluster region (bcr) of chromosome 22 (Heisterkamp, *et al.*, 1985; Shtivelman *et al.*, 1985). In both instances, aberrant patterns of oncoprotein expression are believed to be responsible for induction of transformation (ar-Rushdi *et al.*, 1983; Leder *et al.*, 1983; Shtivelman *et al.*, 1985). To understand the potential role of karyotypic alterations in the etiology of other neoplastic diseases, accurate chromosomal mapping data are essential. In this report, we describe the localization of two human oncogenes, *ros*1 (formerly *mcf*3) and *mas*1, which map to a region on chromosome 6 frequently involved in tumor-specific chromosome rearrangements.

The *ros*1 oncogene, originally designated *mcf*3, was cloned from DNA of the human mammary carcinoma cell line MCF7 using a tumorigenicity assay for activated transforming genes (Fasano *et al.*, 1984; Birchmeier *et al.*, 1986). Nucleotide sequence analysis of the *mcf*3 clones revealed that a major portion of the coding region of this oncogene

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derived from the human homolog of the UR2 strain avian sarcoma virus transforming gene. v-*ros*, but that the oncogene had undergone a rearrangement (Birchmeier *et al.*, 1986). *ros*1 is neither rearranged nor expressed in the original tumor, hence it appears to have become activated as the result of a recombination event during the transfection process. The *mcf*3 transforming gene has been named *ros*1 since other human genes related to v-*ros* or *mcf*3 may exist (Matsushime *et al.*, 1986; Birchmeier *et al.*, in press).

The *ros*1-encoded protein shows a high degree of homology to other oncoproteins which possess tyrosine phosphokinase activity (Birchmeier *et al.*, 1986). Similar structural domains have been defined in the epidermal growth factor receptor (EGFR; Downward *et al.*, 1984) and insulin receptor (IR; Ebina *et al.*, 1985; Ullrich *et al.*, 1985) proteins. A high degree of homology between the amino acid sequence of *ros*1 and IR in the putative substrate binding domain might indicate similar binding specificities for the tyrosine kinase domains of these proteins (Ebina *et al.*, 1985; Ullrich *et al.*, 1985; Birchmeier *et al.*, 1986). Since the *ros*1 encodes a protein with a transmembrane domain, and other oncogenes such as *erb* B1 (Downward *et al.*, 1984; Merlino *et al.*, 1984; Ullrich *et al.*, 1984) and *fms* (Sherr *et al.*, 1985) encode growth factor receptors, it is likely that *ros*1 may also encode a growth factor receptor.

The *mas*1 gene was isolated from DNA of a human epidermoid carcinoma cell line using the cotransfection and tumorigenicity assay (Young *et al.*, 1986). Characterization of *mas*1 clones revealed that this oncogene had also undergone rearrangement during gene transfer. Since *mas*1 is apparently not rearranged nor activated in the original tumor, this rearrangement is likely responsible for activation of its oncogenic potential. Based on its deduced amino acid sequence, the *mas*1 gene product contains seven potential transmembrane domains (Young *et al.*, 1986). The *mas*1 protein is therefore probably an integral membrane protein. Since growth factor receptors have been shown to be membrane-associated, and in more than one instance oncogenic, it is possible that the *mas*1 encoded protein may be a receptor that when activated modulates a critical component in a growth regulating pathway to bring about its oncogenic effects.

Since both *ros*1 and *mas*1 are protooncogenes that can be activated by rearrangement, we undertook to determine their chromosomal map location.

MATERIALS AND METHODS

Cell Lines

Human EBV transformed lymphoblastoid cell lines were grown in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 100 μg/ml kanamycin sulfate, 2 mM L-glutamine. JS and PM are karyotypically normal male and female cell lines, respectively. GM5184, a lymphblastoid cell line with the karyotype: 46,XY,t(6;7)6pter->6q21::7q21->7qter;7pter->7q21.2::6q21->6qter was obtained from the NIGMS Human Genetic Mutant Cell Repsoitory, Camden NJ.

Oncogene DNA Probes

The *ros* DNA probe was isolated using a combination of DNA-mediated gene transfer and a tumorigenicity assay (Fasano *et al.*, 1984). NIH 3T3 cells were cotransformed with DNA isolated from the human mammary carcinoma cell line MCF-7 and the plasmid pKOneo, containing a G148 antibiotic resistence gene. Colonies that incorporated

exogenous DNA were selected by their resistance to the antibiotic. Cotransformed NIH 3T3 cells were injected into nude mice, and DNA obtained from the resulting tumors was used in another round of cotransformation and tumorigenicity assays. Genomic libraries were constructed in the cosmid vector pHC79, and screened as previously reported (Birchmeier *et al.*, 1986). The hybridization probe, pmcf3–5.2, used for the chromosomal mapping studies, contains a unique 5.2 kb EcoRI restriction fragment derived from the 5'-end of the human *ros*1 homolog, subcloned in pUC8.

The *mas* oncogene probe was isolated from a human epidermoid carcinoma cell line using the contransformation-tumorigenicity assay (Fasano *et al.*, 1984; Young, *et al.*, 1986). A cosmid library was constructed using DNA from a nude mouse tumor. Cosmids were screened for tumorigenicity, and one clone, p-*mas*1, was found to induce tumors in nude mice after injection of transfected NIH 3T3 cells. A λgt 10 cDNA library, prepared from poly A^+ mRNA of the nude mouse tumor-derived cell line, MAS-133, was screened by hybridization to pMAS1 DNA, and homologous cDNA sequences were subcloned into pUC8 (Young, *et al.*, 1986). The recombinant plasmid, pMC24, used for in situ chromosomal hybridization, contained a unique 1.7 kb Eco RI cDNA fragment of the human *mas*1 oncogene.

In Situ *Hybridization*

^{125}I-labeled plasmid probes were prepared by nick translation with ^{125}I-dCTP (2000 Ci/mmol, Amersham) according to standard protocols (Rigby *et al.*, 1977). Unincorporated nucleotides were removed by CF11 cellulose chromatography (Szabo *et al.*, 1977). ^{125}I-DNA (average specific radioactivity 5×10^8 dpm/μg) was recovered by ethanol precipitation and redissolved in hybridization mix as previously reported (Rabin *et al.*, 1984).

Metaphase chromosome spreads were prepared from JS and GM5184 cell cultures using standard cytologic techniques. Chromosomes were identified by G-banding prior to hybridization. *In situ* hybridization was performed as described by Rabin *et al.* (1984). Slides were autoradiographed for 7–14 days.

RESULTS

The *ros*1-specific clone, pmcf3–5.2, was used as hybridization probe to map this oncogene to 6q21->q22 (Figure 1). After autoradiography, the distribution of silver grains associated with chromosomes in previously photographed metaphase spreads was plotted on a computer-generated histogram in which a standard idiogram of the human haploid karyotype was divided in units proportional to an average silver grain diameter (0.35μm). A total of 50 hybridized spreads were anlayzed. The extent of background labelling in this experiment was low, averaging less than 1 silver grain per unit chromosome length. Of 270 grains detected on chromosomes, 100 (37%) were located on chromosome 6. Of the chromosome 6-specific silver grains, 81% were localized within the region 6q16->q22 as previously reported (Rabin *et al.*, 1985; Nagarajan *et al.*, 1986).

To confirm and further refine the chromosomal map location of the *ros*1 gene, the pmcf3–5.2 probe was hybridized to metaphase chromosomes prepared from a cell line, GM5184, carrying a reciprocal t(6;7)(q21;q21.2). This cell line was derived from a phenotypically normal male. The distribution of silver grains observed over the normal

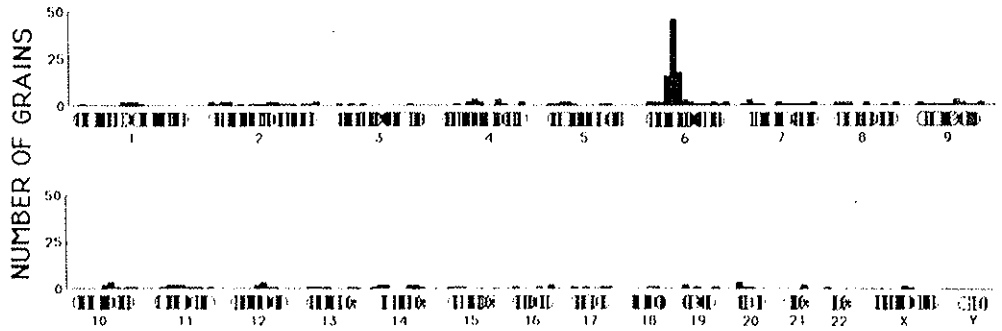

FIGURE 1 Localization of the ROS1 oncogene to chromosome 6 by *in situ* hybridization. Of 270 autoradiographic silver grains, 81(30%) were localized to 6q16-q22.

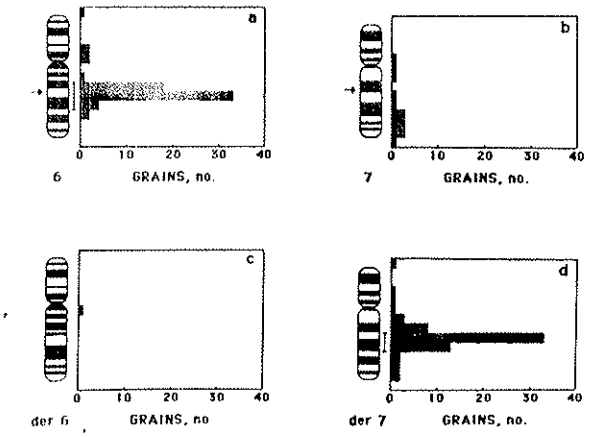

FIGURE 2 Distribution of silver grains over normal and derivative chromosome 6 and 7 homologs of the cell line GM5184 after *in situ* hybridization with the *ros*1-specific probe. GM5184 carries a reciprocal t(6;7)(q21;q21.2), permitting refinement of the *ros*1 map locus to the distal half of 6q21-q22. Arrows indicated respective translocation breakpoints. Brackets indicate *ros*1 locus.

and derived chromosome 6 and 7 homologs, respectively, from 58 spreads was plotted as shown in Figure 2. The pattern of labelling seen over the normal chromosome 6 (Figure 2a) is identical to that observed in Figure 1. The normal chromosome 7 and derivative chromosome 6 display no more than background labelling, respectively (Figure 2b,c). A significant concentration of label is observed on the derivative chromosome 7 in the region corresponding to the distal half of band 6q21->q22 (indicated by the bracket; Figure 2d). These data exclude the region 6q16->q21 proximal to the break point in the translocation chromosome, and refine the map location of *ros*1 to within the distal half of 6q21->q22, a region which represents less than 0.8% of the haploid human genome. *ros*1 therefore maps in close proximity to the *myb* oncogene which has been localized to 6q21-q23 (Dalla-Favera *et al.*, 1982b; Harper *et al.*, 1983; McBride *et al.*, 1983; Janssen *et al.*, 1986). These data rule out allelic identify of *ros*1 with the IR gene, as proposed by Ebina *et al.* (1985), since IR has been mapped to chromosome 19 (Ebina *et al.*, 1985; Yang-Feng *et al.*, 1985).

The human *mas*1 gene was mapped within region 6q24->q27 using the oncogene-specific cDNA probe, pMC24 (Young *et al.*, 1986). *In situ* hybridization and data treatment were performed as detailed above. A total of 75 metaphase spreads were

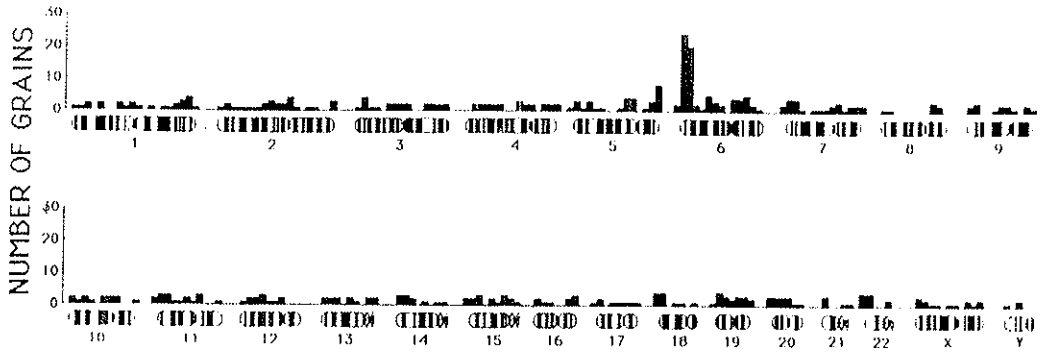

FIGURE 3 Localization of the *mas*1 oncogene to chromosome 6 by in situ hybridization. A total of 418 silver grains were plotted, of which 44(10%) were observed in the region 6q24-q27.

analyzed following autoradiography (Figure 3). The extent of background labelling averaged 1.8 silver grains per unit chromosome length. Of 418 silver grains scored on chromosomes, 75 (18%) were located on chromosome 6. Of the grains present on chromosome 6, 44 (59%) were specifically localized within the region 6q24->q27, which represents approximately 0.8% of the haploid human genome length. These data map the *mas*1 oncogene distal to *ros*1 and *myb*.

DISCUSSION

Correlation of non-random chromosomal rearrangements with distinct types of neoplasias suggests a causal role of karyotypic alterations in malignant transformation. Chromosomal mapping data have implicated a number of genes which control cell growth and differentiation in tumorigenesis by virtue of their localization at rearranged chromosomal loci. Genetic analyses have, at the molecular level, revealed functional associations of such genes with cell transformation events based on their expression in neoplastic tissues. These genes include cellular oncogenes, and genes encoding cell growth factors and their respective membrane-associated receptors. Abberant expression of such genes has been proposed as one mechanism by which normal patterns of cell proliferation may be subverted to permit malignant growth. In this paper, we report the chromosomal mapping of two activated transforming genes, *ros*1 and *mas*1, which are both located in a region on the long arm of chromosome 6 that is frequently rearranged in malignant cells.

Tumor-specific cytogenetic abnormalities of chromosome 6q often involve deletions or translocations (Figure 4; Mitelman *et al.*, 1983; Berger *et al.*, 1985). That rearrangement in this genomic region may play a primary role in malignant transformation was earlier suggested by the localization of the *myb* oncogene to 6q22-q24 (Dalla-Favera *et al.*, 1982b; Harper *et al.*, 1983; McBride *et al.*, 1983; Janssen *et al.*, 1986), and is further supported by our assignment of the *ros*1 oncogene to 6q21-q22. Deletions of the long arm of chromosome 6 are consistently involved in malignant melanomas (Becher *et al.*, 1983; Pathak *et al.*, 1893; Trent *et al.*, 1983). Characteristic chromosomal translocations with breakpoints in the region spanning 6q21-q23 have similarly been reported in mediastinal teratocarcinomas (Oosterhuis *et al.*, 1985), hematologic neoplasias (Mitelman, 1983; Berger *et al.*, 1985), ovarian carcinomas (Wake *et al.*, 1980), and other tumor types. The

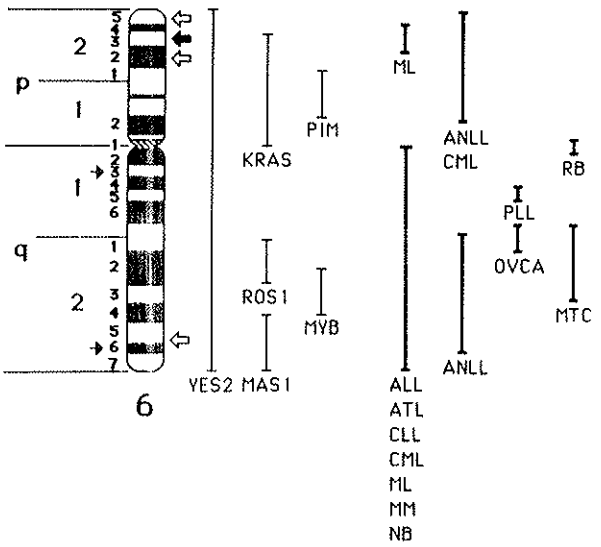

FIGURE 4 Map of chromosome 6 indicating the location of known oncogenes, fragile sites, and chromosomal regions involved in tumor-specific structural rearrangements. Chromosome map locations of known oncogenes are delimited by the light brackets. K-*ras*, *ros*1, *mas*1, *pim*, and *myb* have been sub-chromosomally localized. *yes*2 has been assigned to chromosome 6 but has not been regionally mapped. Regions of chromosome 6 reported to undergo rearrangements characteristic of specific tumor types are indicated by heavy brackets. Neoplasias in which such rearrangements are observed are listed below each bracket, respectively. Chromosomal abnormalities include deletions and translocations. Fragile sites are indicated as follows: heritable, large black arrow; constituitive, large white arrow; common, small black arrow. Classification of fragile sites is as previously reported (Yunis and Soreng, 1984; Glover *et al.*, 1984). Abbreviations: *yes*2, processed Yamaguchi sarcoma virus transforming gene homolog; K-*ras*, Kirsten murine sarcoma virus transforming gene homolog; *pim*, human homolog of the *pim*-1 murine leukemia virus-associated transforming gene; *myb*, human homolog of the avian myeloblastosis virus transforming gene; ALL, acute lymphoblastic leukemia; ATL, adult T-cell leukemia; CLL, chronic lymphocytic leukemia; CML chronic myeloid leukemia; ML, malignant lymphoma; MM, malignant melanoma; NB, neuroblastoma; ANLL, acute non-lymphocytic leukemia; PLL, prolymphocytic leukemia; OVCA, ovarian carcinoma; RB, retinoblastoma; MTC, mediastinal teratocarcinoma.

characteristic t(6;14)(q21;q31) reported in some ovarian carcinomas (Wake *et al.*, 1980) appears cytogenetically to involve the *ros*1 locus on chromosome 6, and the *fos* oncogene site on chromosome 14 (Barker *et al.*, 1984). Whether *ros*1 or *fos* is rearranged in ovarian tumors is currently under investigation. *ros*1 is expressed in some tumors, including glioblastomas (Birchmeier *et al.*, 1986), however, we have not as yet detected cytogenetic alterations of the *ros*1 locus in glioblastoma cell lines.

Localization of *mas*1 to 6q24-q27 similarly suggests that this oncogene may be involved in tumor-specific chromosomal rearrangements. Deletions of 6q observed in some malignant melanomas apparently span the *mas*1 locus (Becher *et al.*, 1983; Pathak *et al.*, 1983; Trent *et al.*, 1983), as do chromosomal abberations in other tumors (Figure 4). The chromosome 6 breakpoint of the characteristic t(6;11)(q27;q23.3) marker associated with acute non-lymphocytic leukemia (ANLL) lies within the *mas*1 locus (Yunis *et al.*, 1981; Lowenberg *et al.*, 1982). This correlation is of interest since the breakpoints of non-random chromosomal rearrangements are often located at fragile sites. Two fragile sites are present within the region to which we have assigned the *mas*1 gene (Figure 4; Yunis and Soreng, 1984; Glover *et al.*, 1984) providing additional circumstantial evidence

in support of the potential involvement of oncogenes and fragile loci in tumorigenesis. The significance of these observations relative to ANLL, however, remains unclear.

Membrane-associated oncoproteins which function as cell surface receptors may induce malignant cell growth via abnormal modes of transmembrane signalling (Heldin and Westermark, 1984; Waterfield 1985). A number of oncogenes, including *erb* B1 (Yamamoto *et al.*, 1983; Downward *et al.*, 1984; Privalsky *et al.*, 1984), *erb* B2 (formerly *neu;* Schecter *et al.*, 1985; Coussens *et al.*, 1985), *trk* (Martin-Zanca *et al.*, 1986), and *ros*1 (Birchmeier *et al.*, 1986) encode proteins which contain potential transmembrane domains and possess tyrosine-specific protein kinase activity characteristic of growth factor receptors. That *ros*1 displays a high degree of amino acid sequence homology with IR, suggested these genes might be allelic. The IR gene had previosuly been mapped to human chromosome 19 (Ebina *et al.*, 1985; Yang-Feng *et al.*, 1985). Localization of *ros*1 to chromosome, as initially reported by Rabin et al. (1985) and confirmed by Nagarajan *et al.* (1986), indicates that *ros*1 and IR are distinct genes since they reside on different chromosomes. These data do not, however, preclude the possibility that *ros*1 might be related to an as yet unidentified cell growth factor receptor.

The *mas*1 gene apparently represents a novel form of membrane-associated oncoprotein. The predicted *mas*1 gene product contains seven hydrophobic domains suggesting it might be an integral membrane protein (Young *et al.*, 1986). While no strong homology between this oncogene and any known DNA or protein sequence has been detected, *mas*1 does share minor protein sequence homology and strong structural similarity with other proteins which span the cell membrane multiple times, including the visual opsins, acetylcholine receptor, and the muscarinic acetylcholine receptor (Birchmeier *et al.*, in press). The *mas*1 oncoprotein may represent a receptor which acts at a critical step in the growth regulatory pathway such as signal transduction or it may function in ion transport as a membrane channel. The tumorigenicity of the activated transforming gene may arise from inappropriate expression of the *mas*1 gene product resulting in deregulation of cell growth.

In this paper, we report the chromosomal map positions of two human oncogenes, *ros*1 and *mas*1, isolated from human tumor DNAs by a combination of transfection and selection for tumorigenic phenotype. The *ros*1 map locus has been refined to the distal half of 6q21-q22, in proximity to *myb*. *mas*1 has been mapped to 6q24-q27. A homolog of the Yamaguchi sarcoma virus (Y73) transforming gene, *yes*2, has also been assigned to chromosome 6, but has not been regionally mapped (Semba *et al.*, 1985). Our data thus bring to at least three the number of oncogenes that have been identified on the long arm of chromosome 6, and may help to explain why this chromosomal region is involved in structural rearrangements in diverse tumor types.

Acknowledgments

We would like to thank Drs. M. Watson and P. Murphy (Yale University) for providing the JS and PM cell lines. We also wish to thank Marilyn Newman, Suzy Pafka, and Vincent Salerno for their excellent technical assistance.

This research was supported by NIH grant GM09966 (to F.H.R.) and by grants from the American Cancer Society, American Business for Cancer Research Foundation, and NIH (to M.W.). M.R. was an NIH Postdoctoral Trainee. C.B. was supported by the Swiss National Science Foundation. D.B. was supported by Institute National de la Sante et de la Recherche Medicale and Phillipe Foundation fellowships. D.Y. is a Cancer Research Institute fellow. M.W. is an American Cancer Society Research Professor.

(Received January 19, 1987; Accepted April 16, 1987)

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