Comprehensive DNA Copy Number Profiling of Meningioma Using a Chromosome 1 Tiling Path Microarray Identifies Novel Candidate Tumor Suppressor Loci

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Abstract

Meningiomas are common neoplasms of the meninges lining of the central nervous system. Deletions of 1p have been established as important for the initiation and/or progression of meningioma. The rationale of this array-CGH study was to characterize copy number imbalances of chromosome 1 in meningioma, using a full-coverage genomic microarray containing 2,118 distinct measurement points. In total, 82 meningiomas were analyzed, making this the most detailed analysis of chromosome 1 in a comprehensive series of tumors. We detected a broad range of aberrations, such as deletions and/or gains of various sizes. Deletions were the predominant finding and ranged from monosomy to a 3.5-Mb terminal 1p homozygous deletion. Although multiple aberrations were observed across chromosome 1, every meningioma in which imbalances were detected harbored 1p deletions. Tumor heterogeneity was also observed in three recurrent meningiomas, which most likely reflects a progressive loss of chromosomal segments at different stages of tumor development. The distribution of aberrations supports the existence of at least four candidate loci on chromosome 1, which are important for meningioma tumorigenesis. In one of these regions, our results already allow the analysis of a number of candidate genes. In a large series of cases, we observed an association between the presence of segmental duplications and deletion breakpoints, which suggests their role in the generation of these tumor-specific aberrations. As 1p is the site of the genome most frequently affected by tumor-specific aberrations, our results indicate loci of general importance for cancer development and progression. (Cancer Res 2005; 65(7): 2653-61)

Introduction

Surgically treated meningiomas are usually benign neoplasms of the meninges lining of the central nervous system and account for 13% to 26% of all intracranial tumors (1). Epidemiologic studies indicate that >90% of meningiomas are asymptomatic (2). The vast majority of these tumors are sporadic; however, 50% of neurofibromatosis type-2 (NF2) patients present with this tumor, making it the second most frequent tumor type associated with this inherited syndrome (3–5). Although meningioma is common in NF2 patients, molecular analyses of sporadic tumors have only revealed aberrations at the NF2 gene, located on chromosome 22, in approximately half of the tumors studied (6–10). A number of studies report the loss of chromosome 22 as the primary consistent aberration in sporadic meningiomas in the absence of mutations in the NF2 gene. This therefore highlights the presence of an alternative genetic etiology, which might be related to other genes located on chromosome 22 (11, 12). In addition, other sites of the genome have also been implicated in meningioma development (13). Among these, deletions of the short arm of chromosome 1 have been established as the second major genetic factor for the initiation/progression of meningioma and correlate with increased morbidity (14, 15). Although previous studies have proposed candidate loci on chromosome 1 (14–16), they have been limited by the low resolution of the techniques applied.

Microarray-based comparative genomic hybridization (matrix-CGH or array-CGH) is a powerful technique, developed to detect DNA copy number changes with high resolution (17, 18). This approach uses differentially fluorescently labeled test and reference DNA, competitively hybridized to DNA spotted on a glass slide. The detection of altered fluorescent ratios between labeled test and reference is indicative of DNA copy number imbalance. This method has previously been employed to address significant questions related to normal and disease associated gene dosage variation in the human genome (19–23). In this study, we have applied a chromosome 1 tiling path genomic microarray for the detection of DNA copy number imbalances in 82 meningioma samples. This array resolves the largest human chromosome (246 Mb) into 2,118 distinct measurement points with an average resolution of ~110 kb. We have identified four candidate sites of genomic imbalance on chromosome 1, which likely harbor gene(s) important for the development and progression of meningioma.

Materials and Methods

Preparation of the chromosome 1 genomic microarray. Large-insert bacterial clones were selected from chromosome 1 sequence-ready clone contigs. Following DNA extraction (24), every cloned DNA sample was fingerprinted (25) and compared with the original restriction fingerprint within the physical map using restriction fingerprinted clones (26). Only clones that generated restriction fingerprint patterns identical to the original data were included on the array. DOP-PCR and amino-linked PCR products were generated and arrayed onto amine-binding slides (CodeLink Activated Slides, GE Healthcare, Fairfield, CT) using a Genomic Solutions
MicroGrid II robot with a spot pitch of 250 μm, as previously described (27, 28). In total, 2,118 BACs/PACs cover chromosome 1 with an average resolution of ~110 kb (Supplementary Table S1). The number of clones distributed across the p and q arms of chromosome 1 is not equal due to the large block of heterochromatic DNA which has been estimated to be ~18 Mb. The clone coverage of the euchromatic region of the q arm therefore contains the same proportional representation as present on the p arm but covers a smaller euchromatic interval. An additional 18 control clones which span chromosome X are also included on the array.

Hybridizations, scanning, and image analysis. Protocols used for DNA labeling, hybridization and post-hybridization processing were done as previously described (19) and are available at http://puffer.genpat.uu.se/chrom_22_array/chrom22.htm. Image acquisition was done using the GenePix 4000B scanner (Axon Instruments, Inc., Union City, CA). Analysis of hybridization intensity was carried out using the GenePixPro 3 image analysis software (Axon Instruments). Ratio of means for the intensities between test DNA and the reference DNA was calculated, as well as the average, SD, and coefficient of variance (CV) of the duplicates for each clone. Clones displaying a CV of >10% between duplicate spots were discarded from further analysis. The fluorescent ratio of the chromosome 1 clones was normalized against the average ratio of the target clones from either retained clones on the 1q or chromosome X. The average normalized interlocus fluorescence ratio (ANIFLR) values were calculated to assess the average fluorescence value for a given number of clones as well as the interlocus variation.

Bioinformatic analysis. Segmental duplications (duplicons) on chromosome 1 were calculated using the program package vmatch (http://www.vmatch.de/). The draft sequence of the human genome was downloaded from National Center for Biotechnology Information as fasta files (build 35.1, released Aug 26, 2004; FTP server: ftp://ftp.ncbi.nlm.nih.gov). The sequence of all chromosomes was preprocessed using the mktree utility of the vmatch package. Preprocessing includes creation of an index structure representing all substrings of the database sequences. Intra-chromosome 1 duplicons as well as duplications between chromosome 1 and other chromosomes were calculated, with a minimum duplcon size of 10 kb and a minimum of 95% sequence identity. The orientation of duplicons (direct or palindromic) was also determined (Supplementary Tables S3 and S4). All duplicons found by vmatch were confirmed by aligning the repeat pairs with the program stretcher from the EMBOSS tools (http://www.rfcgr.mrc.ac.uk/Software/EMBOSS/).

Results

Previous low-resolution cytogenetic and loss of heterozygosity (LOH) analyses have implicated the short arm of chromosome 1 as having a major role in the initiation and/or progression of meningioma (14–16). The rationale of this array-CGH study was to characterize chromosome 1 DNA copy number imbalances in meningioma using a technique with far higher resolution of analysis. We did this investigation using a full-coverage chromosome 1 tiling path microarray, which resolves this chromosome into 2,118 distinct measurement points. In total, we have analyzed 82 meningiomas, and tumors which displayed chromosome 1 aberrations are summarized in Fig. 1. For all subtle genomic imbalances observed (~5 Mb) in tumors, the paired blood-derived DNA was also analyzed. The tumor material was selected based on the previous positive identification of aberrations on chromosome 1 in 157 meningiomas using LOH analysis with 7 to 26 markers (14). The meningioma cases used in this study have been described in previous publications (11, 13, 14, 29, 30). In total, we detected 56 tumors with chromosome 1 aberrations (Fig. 1). Furthermore, we analyzed an additional 10 tumors, which were not included in the previous chromosome 1 study and found two samples with aberrations on this autosome (M34 and M43; Fig. 1). A number of meningiomas also displayed gains (M3 and M13) and losses of chromosome X (M38, M14, and M67).

Deletion of 1p is the most frequent aberration detected. As shown in Fig. 1, the overall picture can be viewed as complex, because of the involvement of different types of aberrations (deletion and/or gain in a single tumor), size (from monosomy 1 to a 3.5-Mb terminal 1p homozygous deletion), and distribution of genetic imbalances on this chromosome. On the other hand, this seemingly complex picture may be simplified to a large extent, if p-arm deletions are considered as the major variable. In every meningioma in which a DNA copy imbalance(s) was detected, loss of 1p was always observed. In accordance with the above, detection of aberrations on the q arm were always accompanied by full or partial loss of 1p. The predominant aberration detected was terminal deletion of the p arm, which ranged in size from whole p-arm deletion (~120 Mb) to the homozygous loss of 35 clones in sample M38 (Figs. 1 and 2). The latter terminal deletion spans ~3.5 Mb of 1p. We analyzed the paired blood DNA from patient 38 and confirmed that the homozygous loss was tumor specific. It is relevant to mention that this tumor is an anaplastic (WHO grade 3) meningioma. Using publicly available databases, we reviewed the gene content within this 3.5-Mb segment and 70 genes were compiled (see Supplementary Table 1). The second most common aberration was the presence of interstitial deletions of 1p (Fig. 1), which were either simple interstitial deletions (M106 and M93), or complex (i.e., an interstitial deletion combined with other aberrations; see for instance M88, Fig. 1). The size of interstitial deletions also varied considerably, between 2 Mb (M88, ID 898-917) and 114 Mb (M93, ID 27-1144).

Although the general view of aberrations displayed in Fig. 1 clearly implicates 1p, the distribution of aberrations supports the potential existence of at least three distinct candidate loci on the short arm of chromosome 1 that could be important in meningioma tumorigenesis. We defined the smallest candidate region (R1, Fig. 1) in the close vicinity of telomere of p-arm (ID 27-35; ~800 kb), based on the detection of the overlapping homozygous deletion of M38 and the large heterozygous interstitial deletion of M93. This segment contains only 13 genes and these are highlighted in Table 1. The second candidate locus (R2, Fig. 1) spans ID 158-344 (~19.3 Mb), which is defined by the centromeric border of the terminal deletion in M117 (ID 344) and the telomeric border of the interstitial deletion in M31 (ID 158). This region is involved in all but one tumor (M79). The third candidate region (R3, Fig. 1) spans ID 432-458 (~3 Mb) and is defined by overlapping heterozygous deletions in tumors M106 and M79. This region has been previously described using low-resolution deletion mapping (14, 31). Thus, our results confirm and further narrow down this locus by ~700 kb. Furthermore, when combining q-arm deletion data from samples M64 and M88, a minimum 1q deleted interval (R4, Fig. 1) was defined between clones ID 2002-2085 (Fig. 3). This region of 83 clones encompasses ~9.5 Mb of 1q. It should be stressed however, that the q-arm aberrations should be seen as secondary events, as these were always detected in conjunction with p-arm aberrations.

Association of segmental duplications and deletion breakpoints in tumors. To understand the mechanisms which may mediate deletions, we analyzed the sequence of chromosome 1 with regard to the presence of segmental duplications (duplicons). These have previously been shown to mediate disease-specific chromosomal alterations through the process of meiotic recombination (32–36). We used relatively relaxed criteria for the detection of
Numerous duplicons were identified and Table 1 summarizes these and their associated clone IDs (see also Supplementary Tables 3 and 4). To simplify the output of analysis, we clustered consecutive clones containing duplicons. The vast majority of detected duplicons were intrachromosomal, with approximately equal distribution of direct and palindromic orientation of these duplicons. In many instances, we observed a positive correlation between the presence of duplicon(s) and the detected deletion breakpoints in tumors. This suggests that duplicons are also involved in generation of chromosomal rearrangement in somatic cells. A recurrent observation in multiple experiments from tumors displaying terminal deletions of 1p and monosomy 1, which is related to the presence of redundant sequences in these clones, was the increased fluorescent ratio of the first four clones at the telomeric end of the p arm (Figs. 2-5). As displayed in Table 1, clones 1 to 4 show a multitude of duplicons that are also present on other autosomes (chromosomes 2, 3, 5, 6, 8, 9, 11, 15, 16, and 19). All other clones contain intrachromosomal duplicons. We therefore concluded that the high redundancy of sequence within these clones was the underlying factor responsible for their increased fluorescence ratios. In contrast, the most telomeric clones of the q arm displayed less similarity to the rest of the genome and therefore done more reliably.

Figure 1. Summary of chromosome 1 DNA copy number imbalances in 58 meningiomas using array-CGH. Each clone represents a distinct measurement point across chromosome 1, which are numbered in groups of 50, according to their position on the chromosome 1 minimal tiling path. The p and q arm of chromosome 1 is covered by 1178 and 940 BAC clones, respectively. Tumor samples (left to right), with their corresponding genomic aberration(s) displayed by the designated colors. Details of the aberration breakpoints are summarized in the table under each of their corresponding graphics. Minimum overlapping regions of deletion (shaded boxes) and labeled R1 to R4. Tumor names that are bold are also shown in other figures. *, clones which are homozygously deleted in tumor M38 (see also Fig. 2). **, three meningioma profiles in which clones display intermittent fluorescent ratio which are consistent with analysis of a mixture of tumor cells (see also Fig. 4). ***, clones which have a diploid level of fluorescence in tumor sample M101 (see also Fig. 5D).
Evidence for genetic heterogeneity of tumor cells. An unexpected finding was the detection of large terminal p-arm deletions in three tumors with two distinctly different levels of fluorescent ratio within the deleted segment of chromosome 1 (M40, M67, and M134; Figs. 1 and 4). Fortunately, one of these three samples originate from a male patient (M134) and we could therefore accurately determine the fluorescent ratios for one copy level, using the values derived from the chromosome X controls. The female-derived tumor M67 displayed a tumor-specific aberration for clones derived from chromosome X, consistent with heterozygous deletion on this chromosome. Deletions on chromosome X have previously been reported for female-derived meningiomas (29, 37–39). Thus, in tumors shown in Fig. 4 there is a clear single DNA copy level of fluorescent ratio on 1p, which is equal to the values derived from the chromosome X clones and therefore represents loss of one copy. There is also a diploid fluorescence ratio across large segments of the q arm in each of the three samples. However, between the two chromosomal

![Figure 2](image-url). Detection of 1p terminal deletions. The p and q arm of chromosome 1 are represented (horizontal lines, top). Vertical line, centromere of chromosome 1. Fluorescence ratio value (y-axis) and clone number according to its position on chromosome 1 (x-axis). Chromosome X controls are plotted after the dotted line. Tumor name (top left) and the number of positively scored loci from chromosome 1 (n) for each experiment (right). This format is also valid for all further array-CGH figures presented.

A. array-CGH profile of M157. A large terminal deletion of the p arm was detected from ID 1-1179 (ANILFR 0.69 \( F \) 0.06) as well as a homozygous deletion (encircled), encompassing clone 1017 was also observed (fluorescent ratio of 0.31).

B. detection of a p-arm deletion in M121. The deletion encompasses ID 1-685 (ANILFR 0.69 \( F \) 0.08).

C. terminal p-arm deletion in M117 was identified from ID 1-344 (ANILFR 0.77 \( F \) 0.05). A gain was observed in clones 1017-18 (ANILFR 1.45 \( F \) 0.07, encircled).

D. heterozygous deletion was identified in M38 from clone ID 1-1136 (ANILFR 0.63 \( F \) 0.09). A terminal homozygous deletion was also detected ID 1-35 (ANILFR 0.33 \( F \) 0.05) as well as a low level gain from ID 1467-1485 (ANILFR 1.16 \( F \) 0.10).

E. enlarged profile of sample M38 displaying the homozygously deleted region.
segments described above, there is yet another interval that displays an intermittent fluorescence ratio. There are at least two possible explanations for the observed profiles. The first may be oligoclonality of these tumors (38). An alternative and perhaps more likely explanation is the progressive loss of different chromosomal segments, which occur at different stages of tumor development and confer a selective advantage to a clone of cells affected with the larger deletion. It is relevant in this context to mention that all three cases are recurrent although not anaplastic meningiomas. As far as we are aware, this is the first study reporting the detection of tumor-specific heterogeneity in DNA derived from primary tumor tissue using array-CGH. Several array-CGH papers have previously reported the reliable detection of DNA copy number imbalances on a heterogeneous cell background in the context of mouse tumor cell lines, human schwannoma, and ovarian cancer (40–42).

Detection of tumor-specific copy number gains and copy number polymorphisms in germ line DNA. Another noteworthy finding was the detection of low-copy number gains across chromosomes 1 and X in the studied series of tumors (Figs. 1 and 5). The regions of gain varied considerably in size and distribution. On chromosome 1, we observed five nonoverlapping segments of gains in four tumors. The maximum copy number detected in the studied tumors was four copies (M159, Fig. 5). Case M101 was unusual as we detected a profile consistent with monosomy 1 with a minute duplication of clones ID 595 to 616, Table 1. Summary of clones from chromosome 1 containing large duplicons

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NOTE: Clone pairs associated with large duplicons. Rows and columns display array ID numbers of the clones used in the hybridization experiments. Only clones that fulfill the criteria used for detection of duplicons are shown. Clones with consecutive IDs were merged into clusters. Filled cells indicate that at least one clone of the cluster displayed in the vertical/horizontal direction harbours at least one duplicon. The characters displayed in the cells indicate the mutual orientation of both duplicons. Shaded cells indicate that the identified duplicon(s) agree with a deletion breakpoint detected in the hybridization experiments. As displayed in the last row/column, clones 1 to 4 show a multitude of duplicons which are also present on other autosomes (chromosomes 2, 3, 5, 6, 8, 9, 11, 15, 16, and 19). All other clones contain intrachromosomal duplicons. For further details, see Supplementary Tables 3 and 4.

Abbreviations: d, direct repeat; p, palindromic repeat.
bringing this segment of 1p to the diploid level. We also studied the paired constitutional DNA for tumors displaying gains and confirmed that these aberrations were tumor specific. In addition, we also observed evidence for tumor-specific gains on chromosome X (M13, Fig. 5 and M3, Fig. 1, array-CGH profile not shown), because our array includes 18 control clones, which are spread across this chromosome.

Although the aim of this study was not to investigate germ line copy number polymorphisms across chromosome 1 in DNA of patients affected with meningiomas, we observed a frequent loss or gain of one to two clones (ID 1016 and 1017) on 1p in the relative vicinity of the centromere. In selected cases, we confirmed these findings by studying constitutional DNA (array-CGH profiles not shown). Circles in Figs. 2, 3, and 5 highlight these clones in tumors M157, M117, M64, and M13. The copy number polymorphism of ID 1016 and 1017 encompass the AMY1A gene (43), which has also recently been shown to be polymorphic in a genome wide array-CGH study of a panel of 55 nonrelated individuals (23).

Interestingly, the occurrence of these germ line copy number polymorphisms can be clearly associated to the presence of duplions in this interval of chromosome 1. Upon bioinformatic analysis, clones ID 1015 to 1018 (RP11-153F1, RP5-1108M17, RP11-508C1, and RP4-724N10) were shown to contain duplions (Table 1). This is a likely example of variation in DNA copy number mediated through meiotic recombination, in contrast to the above-described involvement of duplions in creation of deletions in somatic cells.

### Discussion

This report is the most detailed analysis of the largest human chromosome in a comprehensive series of tumors. We studied >7.5% of the genome with an average resolution of ~110 kb, using a maximum of 2,118 measurement points across chromosome 1. A high average number of positively scored measurement points in all experiments was obtained, ensuring reliability of analysis. The method we applied is superior to previous approaches for the assessment of genomic imbalances (14–16). In this context, there are two major advantages of tiling path array-CGH over previously done analysis. The first is the high resolution of analysis due to the large number of independent measurement points along the whole chromosome. The current resolution of analysis is >50-fold higher than the previously reported chromosome 1 LOH-based studies of meningioma (14–16). The second
advantage is the possibility of independent comparison of tumor and paired constitutional DNA from patients against a pool of DNA from 10 reference female genomes. In this study, we applied this powerful technique with the aim of defining specific region(s) of chromosome 1, which could then be further analyzed for specific genes involved in the disease process. The short arm of chromosome 1 is one of the sites in the human genome that is most frequently affected by tumor-specific aberrations (44). In this broader perspective, our results may indicate genes of importance for development and progression of many other tumor forms. Towards this goal, we defined four candidate chromosome 1 loci (three on 1p and one on 1q), as important for meningioma tumorigenesis. One of these candidate loci (R1, ID 27-35) is already small enough to allow the analysis of a limited number of candidate genes for their role in tumor development. Two interesting candidate genes within the R1 locus that should be studied further are TP73 gene (encoding p53-related protein) and ARHGEF16 (Rho guanine exchange factor 16) gene (Supplementary Table S2). The TP73 gene has been previously studied in meningiomas but only with a limited number of tumors (45, 46). The fact that the homozygously deleted tumor M38 is an anaplastic meningioma suggests that the inactivation of this gene (or other neighboring genes) may be related to the very late stage of meningioma progression. Thus, further studies should address this issue.

Our results warrant further avenues of investigation on multiple levels. The first approach should include analysis of the four candidate loci in a much larger series of tumors and at an even higher resolution. Towards this goal, we intend to apply a nonredundant strategy for construction of genomic arrays.

Figure 4. Array-CGH analysis of a heterogenous population of meningioma cells in two distinct cases. A, detection of a terminal p-arm deletion consisting of two distinct levels of fluorescence ratio in sample M67. The first level of fluorescence ratio spans ID 1-641 (ANILFR 0.52 ± 0.06), while the second encompasses ID 642-1181 (ANILFR 0.66 ± 0.07). B, the first level of fluorescent ratio in sample M134 includes clones ID 1-1153 (ANILFR 0.54 ± 0.07). The second level of fluorescent ratio spans ID 1154-1372 (ANILFR 0.79 ± 0.05). C and D, theoretical array-CGH profiles of two cell populations present in sample M134, which when combined (box) and analyzed using array-CGH, result in the profile shown in B.
which currently allows an average resolution of 2 to 3 kb per data point. Such an array would be instrumental in the detection of minute aberrations likely missed by the clone based approach employed in this study and would thus help to further refine the candidate loci. This should then be followed by screening candidate genes for mutations within the refined candidate loci. Another understudied but potentially very important mechanism is the role of epigenetic changes in meningioma tumorigenesis. The abovementioned 2- to 3-kb average resolution genomic array will also be helpful in concomitant analysis of DNA copy number and epigenetic imbalances, for instance, aberrant methylation of CpG islands in meningioma. This array-CGH study highlights the usefulness of tiling-path arrays for the precise detection of aberration breakpoints across chromosome 1. Although we now have a detailed picture of chromosome 1 aberrations, we still lack a whole genome perspective, which might provide important insights into as unknown defined genomic regions of importance for tumorigenesis of meningioma. Fortunately, the recently reported 32,000 human genome BAC array (49) allows analysis with similar resolution to the chromosome 1 array but extended to the whole genome.

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Figure 5. Detection of DNA copy number gains in four meningiomas on chromosomes 1 and X. A, profile of sample M159 with multiple losses and gains across chromosome 1. The first imbalance is consistent with a single copy loss which includes ID 1-322 (ANILFR 0.75 ± 0.11). A gain was detected from ID 323-520 (ANILFR 1.07 ± 0.28) with a peak value of 1.95 in clone ID 417. The second loss was detected from ID 521-812 (ANILFR 0.78 ± 0.04). The last aberration was a low level gain from ID 813-993 (ANILFR 1.09 ± 0.15), with a peak value of 1.46 in clone ID 900. B, detection of copy number imbalances in sample M11. The terminal deletion spans from ID 1-1170 (ANILFR 0.75 ± 0.06), while a low level gain was detected from ID 1171-1195 (ANILFR 1.12 ± 0.11). C, array-CGH profile of sample M13 displayed a terminal p-arm deletion, which spans part of the q arm from ID 1-1191 (ANILFR 0.74 ± 0.06), with clone 1017 displaying a lower fluorescent ratio (0.49, encircled). A gain of chromosome X was also observed in the control clones (ANILFR 1.42 ± 0.14, rectangle). D, detection of monosomy of chromosome 1 in sample M101 with a region of duplication from ID 595-616 (ANILFR 0.99 ± 0.06).

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