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BCR expression is decreased in meningiomas showing loss of heterozygosity of 22q within a new minimal deletion region

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Abstract Neurofibromin 2 (*NF2*), located on chromosome arm 22q, has been established as a tumor suppressor gene involved in meningioma pathogenesis. In our study, we investigated 149 meningiomas to determine whether there are additional tumor suppressor genes localized on chromosome 22q, apart from *NF2*, that might be involved in meningioma pathogenesis. The LOH analysis on chromosome 22q identified two regions of deletion: the first one, which is limited to the *NF2* gene locus, and the second one, which is outside this location. The new minimal deletion region (MDR) included the following genes: *BCR* (breakpoint cluster region), *RAB36* (a member of *RAS* oncogene family), *GNAZ* [guanine nucleotide binding protein (G protein), α-z polypeptide], and *RTDR1* (rhabdoid tumor deletion region gene 1). The expression levels of all these genes, including *NF2*, were subsequently analyzed by quantitative real-time polymerase chain reaction. We observed a significantly lowered expression level of *NF2* in meningiomas with 22q loss of heterozygosity (LOH) within *NF2* region compared to the one in meningiomas with 22q retention of heterozygosity (ROH, $P<0.05$). Similarly, *BCR* showed a significantly lowered expression in meningiomas with 22q LOH within the new MDR compared to cases with 22q ROH ($P<0.05$). Our data, together with the already published information considering BCR function suggest that *BCR* can be considered as a candidate tumor suppressor gene localized on chromosome 22q which may be involved in meningioma pathogenesis.

1. Introduction

The *NF2* (neurofibromin 2) gene, localized on chromosome 22q, has been found to be mutated in meningiomas and thus has been established as a tumor suppressor gene [1]. To date, however, it still remains unclear whether it is the only tumor suppressor gene located on 22q that is involved in meningioma pathogenesis [2,3]. The frequency of 22q deletions was observed to be higher than that of *NF2* mutations, suggesting that there might be other tumor suppressor genes localized on this chromosome [3]. Possible candidates include *MN1* and *INI1* [3,4]. *INI1* mutations were found in a small group of meningiomas [5], and the importance of *MN1* has not yet been confirmed. Moreover, a new minimal deletion region on 22q, not including the *NF2* gene, was recently described in meningiomas [6]. In this paper, we aimed to investigate whether there are additional tumor suppressor genes localized on 22q apart from *NF2*. To do so, we used the loss of heterozygosity (LOH) analysis to identify a new minimal deletion region (MDR) on 22q and subsequently analyzed the expression levels of *NF2* and genes located in the MDR in a set of meningiomas.

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2. Materials and methods

2.1. Tumor samples

The study included 149 cases of meningioma diagnosed and typified according to the World Health Organization criteria for classification of brain tumors [7]. The group consisted of 98 (65.8%) females and 51 (34.2%) males ranging in age from 22 to 91 years (mean age, 56 years). The tumor localizations were distributed as follows: 147 intracranial and 3 spinal. The tumors series included 124 (83%) benign (WHO grade I) and 25 (17%) atypical (WHO grade II) meningiomas. There were 44 transitional, 33 fibrous, 27 meningothelial, and 12 psammomatous subtypes identified in this group. Within the tumor samples, there were 139 primary and 13 recurrent tumors. The clinical data indicated that none of the patients suffered from neurofibromatosis type 2.

2.2. DNA and RNA extraction

The isolations were performed using snap-frozen tissues stored at −80°C. DNA was isolated from paired tumor tissues and blood samples by a standard proteinase K digestion and a phenol/chloroform extraction method. Total cellular RNA was isolated from tumor tissues using Nucleo Spin RNA/Protein kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol.

2.3. Microsatellite analysis and identification of the MDR

The specimens were examined for LOH using paired tumor specimens and corresponding peripheral blood samples. The primers were selected by use of the National Center for Biotechnology Information database and obtained from MWG-Biotech AG (Martinsried, Germany). The forward primers were 5′-end fluorescent-labeled. Polymerase chain reaction (PCR) was performed in thermocycling conditions established individually for each pair of primers. PCR products were visualized with a LiCor automatic sequencer. LOH was judged to be present if the allelic signal intensity of the tumor sample was reduced by at least 50% relative to the corresponding allele in the patient's control DNA.

In cases that showed retention of heterozygosity (ROH) on 22q, additional markers on 1p, 9p, 10q, 14p, and 18q were used to estimate the degree of contamination by normal nonmalignant cells (see the next paragraph). The identification of MDR was based on LOH of the loci that span common deletion in several tumors and retention of heterozygosity of adjoining markers at both the boundaries. Markers used for minimal deletion region detection are presented in Table 1 and Fig. 2.

2.4. Calculating the percentage of nontumor cells contaminating tumor samples

The contamination of the samples with nontumor cells was initially estimated by a pathologist, and then LOH analysis was used to more precisely calculate the percentage of nontumor cells. To accomplish that, standardization curves showing ratios between two alleles of microsatellite markers were obtained. Two glioblastomas presenting LOH of 1p, 9p, 10q, 18q, and 22q were cloned. DNA from cloned cells (free of contamination of DNA without LOH) was obtained. DNA was simultaneously isolated from the peripheral blood leukocytes of the same patients. DNA isolated from cloned glioblastoma cells, and peripheral blood cells were mixed in the following proportions of non-tumor versus tumor DNA: 0/100, 1/99, 2/98, 4/96, 6/94, 8/92, 10/90, 20/80, 40/60, and 100/0 (%). The DNA was then amplified with LOH markers of 1p, 9p, 10q, 18q, and 22q chromosomes. The PCR products were electrophoresed (Fig. 1c), and the intensity of bands corresponding to the two alleles was measured spectrophotometrically. The ratios of band intensities were calculated for every proportion of nontumor versus tumor cells to obtain standardization curves.

Table 1
Microsatellite markers used in the study

Chromosome Arm	Microsatellite Marker
1p	D1S508, D1S199, D1S197, D1S162
	D1S2734, D1S2720, D1S429
9q	D9S162
	D9S156, D9S1748, D9S319
10q	D10S1709, D10S209, D10S587
	D10S197
18p	D18S481
22q	D22S303, D22S257, D22S1163, D22S1150
	D22S449, D22S258, D22S446, D20S608

2.5. Quantitative real-time PCR

For real-time PCR purposes, only samples with negligible nontumor cell contamination (percentage of nontumor cells lower than 4%), were selected based on LOH analysis. Contaminating residual genomic DNA was removed by DNase I digestion. An aliquot containing 1 mg of isolated RNA was reverse-transcribed into single-stranded cDNA in a final volume of 40 μL using QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA).

Real-time quantitative PCR was performed on a Rotor Gene 6000 (Corbett, Life Sciences, Sydney, Australia) instrument using the SYBR Green Master Mix (Applied Biosystems, Foster City, CA). The primer sequences used for amplification of *NF2* and genes localized within the MDR are shown in Table 2. Each sample was amplified in triplicate in a reaction volume of 10 μL containing 50 ng cDNA, 1× SYBR Green Master Mix, and forward and reverse primers (35 ng each). The cycling conditions were as follows: 3 minutes at 95°C (polymerase activation) followed by 40 cycles of 15 seconds at 95°C (denaturation), 45 seconds at 59°C (annealing), and 1 minute at 72°C (extension). *GAPDH* was used as a reference gene for the