Complex humoral immune response against a benign tumor: Frequent antibody response against specific antigens as diagnostic targets

Nicole Comtesse*, Andrea Zippel*, Sascha Walle*, Dominik Monz*, Christina Backes*, Ulrike Fischer*, Jens Mayer*, Nicole Ludwig*, Andreas Hildebrandt†, Andreas Keller†, Wolf-Ingo Steudel‡, Hans-Peter Lenhof†, and Eckart Meese*§

*Department of Human Genetics, Medical School, University of Saarland, Building 60, 66421 Homburg/Saar, Germany; ‡Department of Neurosurgery, Medical School, University of Saarland, Building 90, 66421 Homburg/Saar, Germany; and †Department of Bioinformatics, University of Saarland, Building 36.1, 66041 Saarbrücken, Germany

Edited by George Klein, Karolinska Institutet, Stockholm, Sweden, and approved May 16, 2005 (received for review January 17, 2005)

There are numerous studies on the immune response against malignant human tumors. This study was aimed to address the complexity and specificity of humoral immune response against a benign human tumor. We assembled a panel of 62 meningioma-expressed antigens that show reactivity with serum antibodies of meningioma patients, including 41 previously uncharacterized antigens by screening of a fetal brain expression library. We tested the panel for reactivity with 48 sera, including sera of patients with common-type, atypical, and anaplastic meningioma, respectively. Meningioma sera detected an average of 14.6 antigens per serum and normal sera an average of 7.8 antigens per serum ($P = 0.0001$). We found a decline of seroreactivity with malignancy with a statistical significant difference between common-type and anaplastic meningioma ($P < 0.05$). We detected 17 antigens exclusively with patient sera, including 12 sera that were reactive against KIAA1344, 9 against natural killer tumor recognition (NKTR), and 7 against SRY (sex determining region Y)-box2 (SOX2). More than 80% of meningioma patients had antibodies against at least one of the antigens KIAA1344, SC65, SOX2, and C6orf153. Our results show a highly complex but specific humoral immune response against a benign tumor with a distinct serum reactivity pattern and a decline of complexity with malignancy. The frequent antibody response against specific antigens offers new diagnostic and therapeutic targets for meningioma. We developed a statistical learning method to differentiate sera of meningioma patients from sera of healthy donors.

meningioma

Meningioma arises from the coverings of the brain and spinal cord and constitutes ≈20% of primary intracranial tumors with an average incidence of 6 per 100,000 in the population (1). In general, meningioma is a slowly growing benign tumor corresponding to grade I in the World Health Organization (WHO) classification. Atypical meningioma, which are characterized by increased cellularity and increased mitotic activity, account for ≈8% (WHO grade II) and anaplastic meningioma (WHO grade III) for ≈2% of this tumor type (1). Loss of chromosome 22, deletion of the short arm of chromosome 1, and loss of chromosome 14 are frequent cytogenetic changes (2–6). The neurofibromatosis type 2 (*NF2*) gene on chromosome 22q appears to be a tumor suppressor gene in meningioma. However, 40% of meningioma show no mutations of the *NF2* gene, indicating that additional genes are involved in the tumorigenesis (7, 8).

Several studies provided evidence that meningioma is capable of inducing a humoral immune response in the patient. Previously, we reported identification and cloning of several immune reactive antigens expressed in meningioma, including the meningioma-expressed antigens MGEA6/11 and MGEA5, the latter of which appears to be a hyaluronidase (9–12). Antibodies against MGEA6/11 occur in >41% of sera from meningioma patients and are likely attributed to overexpression of MGEA6/11 protein in tumor cells (12).

Immunogenic tumor-associated antigens have been reported for a large variety of malignant tumors, including melanomas and colon cancer. The finding of immunogenic antigens in meningioma leaves several questions. Are benign tumors associated with a frequent antibody response? Is there a complex antibody response? Is there a specific antibody response? Is this response associated with specific genetic features of the tumor? Do these immunogenic antigens share common features like specific sequence motives? To answer these questions, we choose meningioma, a generally benign tumor that is extensively characterized by genetic means. We assembled a panel of 62 immunogenic antigens that lays the ground for a comprehensive analysis of the humoral immune response in meningioma patients.

Materials and Methods

cDNA Expression Library Construction. Human Fetal Brain Poly(A)+ RNA (BD Biosciences, Franklin Lakes, NJ) was used to construct a cDNA expression library in ZAP Express vector arms of lambda phage (Stratagene) as described in ref. 9.

Tumor Tissues and Blood Sera. Informed consent was obtained from patients for use of tumor samples and blood sera. Before surgery, patients underwent anticonvulsant but no immunosuppressive treatment regimen. Tissue samples were frozen in liquid nitrogen immediately after surgery and were stored at −70°C. Blood serum was isolated from 10-ml samples by using serum gel monovettes and was stored at −70°C.

Serum Preabsorption. Before use in immunoscreening, serum was preabsorbed five times against *Escherichia coli* XL1 Blue MRF′ and also five times against bacteria lysed by nonrecombinant ZAP Express phages as described in ref. 9. The preabsorbed serum was diluted to a final concentration of 1:100 in 1× Tris-buffered saline/0.5% (wt/vol) dry milk/0.01% thimerosal.

Immunoscreening of Recombinant Proteins (Standard SEREX). A total of 12 sera from meningioma patients were combined in three groups, each containing four sera from meningioma patients with tumors of the same WHO grade. Final concentration of each serum in the pool was 1:100. *E. coli* XL1 Blue MRF′ cells were transfected with the fetal brain cDNA library and plated at an density of ≈10,000 plaque-forming units per plate as described in ref. 9. Recombinant protein expression was induced and antigen–antibody complexes were detected with alkaline-phosphatase-

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: NKTR, natural killer tumor recognition; WHO, World Health Organization.

§To whom correspondence should be addressed. E-mail: hgemee@uniklinik-saarland.de.

conjugated goat-anti-human IgG antibody (DIANOVA), followed by incubation with 0.005% 5-bromo-4-chloro-3-indolyl phosphate and 0.01% *p*-nitro blue tetrazolium chloride in 1× color developing solution as described in ref. 9.

DNA Sequencing. Sequencing of cDNA clones was performed by using the SequiTherm EXCEL II DNA sequencing kit (Biozym Scientific, Oldendorf, Germany) and the LI-COR 4000L automated sequencer. Primers used for sequencing were the M13 uni (−21) 5′ TGT AAA ACG ACG GCC AGT 3′ and M13 rev (−29) 5′ CAG GAA ACA GCT ATG ACC 3′.

SEREX and Serological Spot Assay of Positive Clones. Screening of positive clones was either by conventional SEREX (13) or by serological spot assay that was performed with minor variations as described in ref. 14. In brief, nitrocellulose membranes were precoated with a layer of NZCYM/0.7% agarose/2.5 mM isopropyl β-D-thiogalactoside and placed on a reservoir layer of NZCYM/0.7% agarose in a 86 × 128 mm Omni Tray (Nalge Nunc). Forty microliters of monoclonal phage at a concentration of ≈5,000 plaque-forming units/μl were incubated with 40 μl of exponentially growing *E. coli* XL1 Blue MRF', and 0.7-μl aliquots were spotted on the precoated nitrocellulose membranes by using the TSP 96-pin replication system (Nalge Nunc). Membranes were incubated overnight at 37°C. The agarose film was then removed from the membrane, and the filters were processed for reactivity with individual serum samples at 1:100 dilution. A total of 45 different antigens and three times the cDNA library as control was spotted in duplicate per nitrocellulose membrane. To provide an unbiased test, we compared the spot intensity with the corresponding negative control. A Gaussian sample of 50–100 points around the center of the spot was taken, and the intensity was averaged over the sample. A spot was classified as positive if the sampled intensity for both spots of an antigen was significantly higher than the intensity of the negative control.

RNA-Isolation and RT-PCR. RNA was isolated by using TRIzol Reagent (Invitrogen) according to the manufacturer's instructions and subsequently purified by using RNeasy Kit (Qiagen). RNA was treated with 5 units/μg DNaseI (Roche Applied Science, Mannheim, Germany) for 25 min at 37°C. Absence of DNA was evaluated by AluPCR with A1S primer (5′ tca tgt cga cgc gag act cca tct caa a 3′). Reverse transcription was performed by using oligo(dT)$_{12–18}$ primers (Invitrogen) and Omniscript RT Kit (Qiagen). Primer sequences used for PCR were as follows: GAPDH for (5′ggaaggtgaaggtcggagt3′), GAPDH reverse (5′atcacgccacagtttccc3′), SOX2 EST forward (5′cctccgggacatgatcag3′), SOX2 EST reverse (5′ttctccccctccagttc3′), SOX2 genomforward (5′agtctgccgagaatccatgt3′), SOX2 genomreverse (5′tgctttcttggctgagcac3′), NKTR RT forward (5′agctactctagaagtcggagc3′), NKTR RT reverse (5′cgattataacttctgcctcgg3′), KIAA1344 RT forward (5′tgattttgttcagtgatggcactg3′), and KIAA1344 RT reverse (5′gatatgattttctagtcctgcttc3′).

Computational Analysis. Statistical analyses were done by using GRAPHPAD INSTAT (GraphPad, San Diego). Reactivity patterns of meningioma sera were analyzed by using several statistical learning methods including linear discriminant analysis, quadratic discriminant analysis, and support vector machines (15). Data for computational analysis were extracted from different databases. Information on chromosomal localization, protein function, and subcellular localization was retrieved from National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and GeneCards (http://bioinfo.weizmann.ac.il/cards/index.shtml), the KEGG PATHWAY Database (www.genome.jp/kegg/pathway.html) for information on pathways, and SMART (http://smart.embl-heidelberg.de) for information on domains. Prediction of cleavage sites for granzyme B was done by GRABCAS, a recently developed prediction tool for granzyme B and caspase cleavage sites (16) that is based on experimentally determined substrate specificities (17).

Results

Identification of Antigens Reactive with Sera from Meningioma Patients. Previously, we reported identification, cloning, and characterization of several immunogenic antigens in meningioma (9–12, 18). Although these studies were largely based on single serum analysis, we now set out to arrive at a more complete picture of the antibody response in meningioma patients. We performed conventional SEREX screening by using 12 sera from patients with meningioma. According to WHO grading, we used four sera of patients with common type meningioma (WHOI), four sera of patients with atypical meningioma (WHOII), and four sera of patients with anaplastic meningioma (WHOIII) for screening of a fetal brain expression library. Use of the fetal brain library enabled analysis of a wide range of cDNAs that are not restricted to a certain tumor type or tumor stage. We combined the sera of each WHO grade into one pool to screen 300,000 clones with each of the sera pools. Positive cDNA clones were confirmed in a second round of screening, excised *in vivo*, and sequenced. In total, we identified 115 positive clones that represent 41 different sequences encoding potential meningioma-related antigens. In detail, serum pool I derived from patients with common type meningioma identified 26 different sequences, pool II derived from atypical meningioma patients identified 9, and pool III derived from anaplastic meningioma patients identified 7 different sequences. Antigen TNKS2 (TRF1-interacting ankyrin-related ADP-ribose polymerase 2) was identified both with pool I and with pool II sera.

Frequency of Antibody Response. To analyze the frequency of the antibody response, we assembled a panel of 62 meningioma-derived antigens. The panel includes antigens found in the above-mentioned screening of a fetal brain expression library and antigens that we previously reported to react with meningioma sera (refs. 9, 10, and 18; unpublished data). The panel contained 53 known proteins and 9 uncharacterized gene products [KIAA clones, FLJ series (hypothetical proteins), and chromosomal ORFs] as summarized in Table 4, which is published as supporting information on the PNAS web site. We tested the panel for reactivity with 48 sera, including 24 sera of meningioma patients and 24 healthy control samples. The 24 patient sera included 8 sera of patients with common-type, atypical, and anaplastic meningioma each. The analysis was done by SEREX or by a serological spot assay that allows screening in a 96-well format (13, 14). Fig. 1 shows an exemplary result of the spot assay.

First, we compared the overall frequency of the antibody response against the 62 antigens between meningioma sera and normal sera. Meningioma sera detected between 3 and 21 antigens per serum with an average of 14.58 antigens per serum. Normal sera detected between 3 and 13 antigens per serum with an average of 7.75 antigens per serum (Fig. 2). These data confirm our previous results that indicated an elevated antibody response in meningioma patients in comparison with individuals without known disease ($P = 0.0001$, unpaired t test with Welch correction). In addition, our data show a relatively frequent antibody response in normal sera with 13 (54.2%) of the normal sera reacting against at least eight antigens.

We further compared the antibody response in meningioma of different grades. In general, we found a decline of seroreactivity with malignancy. On average, sera of common-type meningioma were reactive against 17 antigens, sera of atypical meningioma were reactive against 15 antigens, and sera of anaplastic meningioma were reactive against 11 antigens (Fig. 2).

Specificity of the Antibody Response. Using 48 sera, the spot assay revealed reactivity against 57 of the 62 antigens. The remaining five antigens did not react with any of the sera. Of the 57 antigens, 17 were specifically detected with sera of meningioma patients. More

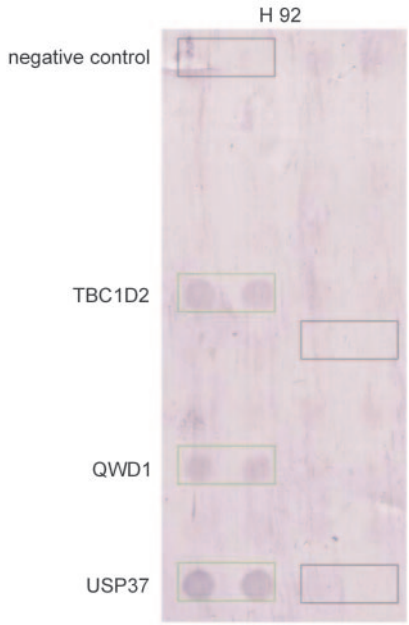

Fig. 1. Representative result of a spot assay. Each clone is spotted in duplicate. Tumor serum H92 detects three clones indicated by green frames. The library that serves as control and is indicated by gray frames is spotted three times on the membrane.

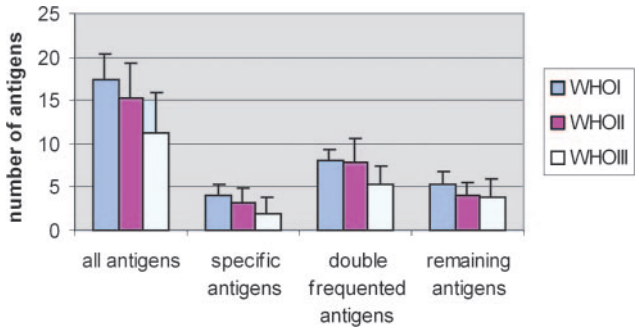

Fig. 2. Serum reactivity against antigens previously identified by meningioma sera. Mean reactivity and standard deviation of sera derived from meningioma patients ($n = 24$) and healthy controls ($n = 24$) against 62 antigens are 7.75 ± 3.01 and 14.58 ± 4.79. Mean reactivity of sera derived from patients with common type meningioma (WHOI), atypical meningioma (WHOII), and anaplastic meningioma (WHOIII). Reactivity was tested against the complete set of antigens, meningioma-specific antigens, antigens that were found twice as frequent with sera of meningioma patients as with sera of healthy donors, and the remaining antigens. The difference between common-type and anaplastic meningioma was statistically significant for the combined sets of antigens that were specific for meningioma and were found twice as frequent in meningioma as in normal controls ($P < 0.05$). The difference between common-type and anaplastic meningioma was statistically not significant for the set of the remaining antigens.

than 50% of these meningioma-specific antigens were represented by full-length ORFs. Two antigens were specifically detected with sera of healthy donors, and 38 antigens were detected both with sera of tumor patients and healthy donors (Fig. 3*A*). The majority of these 38 antigens showed a more frequent antibody response in sera of meningioma patients than in sera of healthy donors (Fig. 3*B*).

The 17 antigens that were specifically reactive with sera of meningioma patients include three sequences encoding hitherto uncharacterized gene products, two previously described meningioma-expressed antigens (MGEA11 and MGEA5s), two proteins involved in neurogenesis (SOX2 and PAFAH1B1), the synaptonemal complex protein and nuclear autoantigen SC65, three immunomodulatory factors (NKTR, TBC1D4, and NSEP1), a ubiquitin specific protease (USP37), a progesterone-induced blocking factor (C13orf24), a tankyrase (TNKS), a Rho GTPase activating protein (ARHGAP18), a serin-threonin kinase (MAP4K4), and NIT2, probably involved in nitrogen metabolism.

Of the 17 antigens exclusively detected with tumor patient sera, 15 antigens were reactive with two or more patient sera. In detail, 12 patient sera (50%) were reactive against KIAA1344, nine patient sera (37.5%) were reactive against NKTR, and seven patient sera (29%) were reactive against SOX2. Table 1 provides an overview of the 17 antigens and their reactivity with meningioma sera.

As mentioned above, 38 antigens showed reactivity with sera of tumor patients and healthy donors, including 20 antigens that were at least twice as frequent reactive with sera of meningioma patients than with sera of healthy donors. Of these antigens, MGEA6, SLC6A3, KIAA0999, ANK2, and DLD were reactive with 1 control serum each. SFRS11 and CDH12 were both reactive with 2 control sera. MGEA6 was detected with 10 tumor sera, SLC6A3 and KIAA0999 were both detected with 8 tumor sera and ANK2, DLD, SFRS11, and CDH12 were each detected with 6 tumor sera (Table 2).

Frequency of the Antibody Response in Meningioma of Different Grades. As indicated above, sera of high-grade meningioma patients show a reduced frequency of antibody response against the panel of 62 antigens. We found a comparable decrease when considering subgroups of antigens. We analyzed the frequency of reactivity against the 17 antigens found exclusively in meningioma patients. On average, patients with common-type meningioma showed antibodies against 4.0 antigens, patients with atypical meningioma against 3.3 antigens, and patients with anaplastic meningioma against 1.9 antigens (Fig. 2). Three sera from patients with anaplastic meningioma did not show reactivity against any of the 17 antigens. We also found the reduced frequency of reactivity against the 20 antigens that are twice as frequently found with sera of meningioma patients than with sera of healthy donors (Fig. 2). The difference between common-type and anaplastic meningioma was statistically significant for the combined sets of meningioma-specific antigens and antigens that were found twice as frequent with meningioma as with sera of healthy donors ($P < 0.05$; Kruskal–Wallis test with Dunn's post test). The difference between common-type and anaplastic meningioma was statistically not significant for the remaining antigens (Fig. 2).

Likewise, reduced serum reactivity is obvious for specific antigens. A total of seven antigens showed reactivity with sera of common-type and/or atypical meningioma patients but not with sera of patients with anaplastic meningioma. For the most frequent antigen KIAA1344, frequency of the antibody response decreased with malignancy grade; antibodies against KIAA1344 were found in 75% of sera of patients with common-type meningioma, in 50% of atypical meningioma, and in 25% of anaplastic meningioma (Table 1).

Combined Specificity and Frequency of the Antibody Response. We further asked whether combinations of antibodies against immunogenic antigens preferentially occur in meningioma sera. Three patients, namely H78, H392, and H82, showed serum antibodies against KIAA1344, NKTR, and ANK2. Two of them were patients with common-type meningioma, and one patient had an atypical meningioma. Additionally, five patients (H375, H365, H444, H108, and H386) showed serum reactivity against two of these antigens without response against the third. In total, 8 of 24 (33.3%) meningioma patients, including three patients with common-type meningioma, three with atypical meningioma, and two with anaplastic meningioma had serum antibodies against at least two of the three antigens (Table 5, which is published as supporting information on the PNAS web site). The observed frequency (33.3%) of the

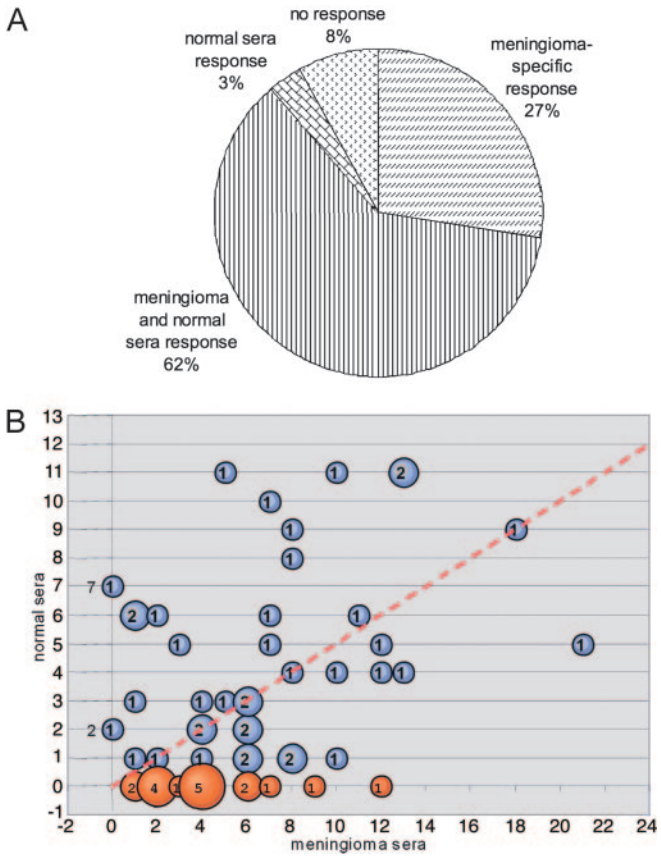

Fig. 3. Number of antigens that reacted with serum antibodies of meningioma patients and healthy individuals. (*A*) Overall percentage of antigens that react with meningioma sera only, with sera of healthy individuals only, and both with sera of meningioma and healthy individuals. (*B*) Frequency of antigens that show reactivity against meningioma sera and/or sera of normal individuals. The size of the circle reflects the number of antigens reactive against a given number of sera. Meningioma specific antigens are indicated in red. As an example, we found five antigens that reacted with four meningioma sera but not with any normal sera.

combined occurrence of at least two of these antibodies is in the range of the expected frequency of 31.8%. However, the observed frequency of the combined occurrence of the antibody response against KIAA1344, NKTR, and ANK2 is 12.5%. This frequency is 2.7 times higher than the expected frequency of 4.7% (Table 3).

Besides the analysis of combined occurrence of antibody response, we asked for the minimal number of tumor-related antigens necessary to detect immune responses in a maximum number of patients. Only 4 antigens, namely KIAA1344, SC65, SOX2, and C6orf153, are sufficient to include 20 of 24 (83%) of the meningioma patients. A combination of KIAA1344, NKTR, SOX2, and C6orf153 detected 19 of 24 (79%) of the meningioma patients.

We investigated the possibility to predict whether a given serum is a meningioma serum based on the antigen pattern detected in the serum. Of several statistical learning methods (linear discriminant analysis, quadratic discriminant analysis, support vector machines, and Bayesian) (15), the Bayesian approach yielded best results. Let S be the set (panel) of all 62 meningioma-derived antigens. Each antigen in S has an index s. For each antigen s in our antigen set S, we define $P(s)$ as the following ratio:

$$P(s) = \frac{P_M(s)}{P_N(s)}, \qquad [1]$$

where $P_M(s)$ is the probability of an antibody response against s in meningioma sera and $P_N(s)$ is the probability of an antibody response against s in normal sera. If the probability $P_N(s)$ is zero, which happens for meningioma specific antigens, we change the value of $P_N(s)$ to $1/25$, i.e., we assume that we may detect an antibody reaction against s in the "next" experiment with a normal serum. For each serum, we identified an antigen pattern A that can be described by the set of indices of the detected antigens. Given this antigen pattern A, we define the following product

$$P(A) = \prod_{s \in A} P(s), \qquad [2]$$

representing a measure that the serum is a meningioma serum, i.e., the higher the value of $P(A)$ the more likely the serum is a meningioma serum. Our learning algorithm classifies a serum as meningioma serum if $P(A)$ is larger than a given threshold t. Sera with $P(A)$ smaller than t are classified as nonmeningioma sera. The threshold t can be modified to obtain higher specificity, defined as

$$\frac{\text{Number of true negatives}}{\text{number of false positives} + \text{number of true negatives}}, \qquad [3]$$

or to obtain higher sensitivity, defined as

$$\frac{\text{Number of true positives}}{\text{number of true positives} + \text{number of false negatives}}. \qquad [4]$$

We tested the approach by using "Leave One Out Cross Validation" (15). The results of our method, presented in Fig. 4, indicate that the antigen pattern detected with sera allows differentiation between sera from meningioma and sera from healthy donors.

Table 1. Antigens exclusively detected with meningioma patient sera

Name	Meningioma total (24 sera)	Meningioma grade: Common type (8 sera)	Atypical (8 sera)	Anaplastic (8 sera)
KIAA1344	12	6	4	2
NKTR	9	4	2	3
SOX2	7	3	2	2
TBC1D4	6	4	1	1
USP37	6	0	3	3
C6orf153	4	2	2	0
MGEA11	4	4	0	0
NSEP1	4	0	4	0
SC65	4	2	1	1
C13orf24	4	2	2	0
NIT2	3	0	2	1
ARHGAP18	2	2	0	0
MGEA5s	2	1	1	0
TNKS	2	1	1	0
FLJ10747	2	0	1	1
MAP4K4	1	0	0	1
PAFAH1B1	1	1	0	0

mRNA Expression of Selected Antigens in Meningioma Tissues and Normal Brain. We analyzed mRNA expression of meningioma-antigen encoding genes including *KIAA1344*, *NKTR*, and *SOX2* in 20 meningioma tissues of different WHO grades and in normal brain by semiquantitative RT-PCR. Representative results are shown in Fig. 5, which is published as supporting information on the PNAS web site. Whereas *KIAA1344* and *NKTR* expression was found in the majority of samples, strong *SOX2* expression was detected in only two of five anaplastic meningioma. The overall expression level did not correlate with the antigen response.

Table 2. Antigens that were detected only in one or two sera of healthy donors and that were at least twice as frequent detected in sera of meningioma patients than in sera of healthy donors

Name	Meningioma total (24 sera)	Meningioma grade			Normal (24 sera)
		Common type (8 sera)	Atypical (8 sera)	Anaplastic (8 sera)	
MGEA6	10	5	2	3	1
KIAA0999	8	2	4	2	1
SLC6A3	8	3	3	2	1
ANK2	6	2	3	1	1
DLD	6	2	3	1	1
SFRS11	6	1	3	2	2
CDH12	6	3	3	0	2

Computational Analysis of Positive Clones. We analyzed 37 antigens that were exclusively found with meningioma sera or that were at least twice as frequent in sera of meningioma patients as in sera of healthy donors. Our analysis included chromosomal localization of the encoding gene, subcellular localization of the protein, protein functions, association with cellular pathways according to the Kyoto Encyclopedia of Genes and Genomes, functional domains, putative granzyme B cleavage sites, and coiled-coil domains (see Table 6, which is published as supporting information on the PNAS web site). For comparison, we used a set of 100 randomly selected protein sequences taken from the National Center for Biotechnology Information database. Of the 37 proteins that reacted with meningioma sera, 16 proteins (43.24%) showed a coiled-coil domain compared with only 16% of the proteins from the random set. Likewise, we found a difference in the percentage of proteins with a predicted granzyme B cleavage site. Of the 37 meningioma antigens, we found also 16 proteins (43.24%) with a predicted granzyme B cleavage site. Of the random set, we found 23% proteins with a predicted granzyme B cleavage site. Analysis of the chromosomal origin of the antigens indicated preferential mapping on several chromosomes and chromosome regions when compared with the overall distribution of the National Center for Biotechnology Information chromosome annotations (Table 7, which is published as supporting information on the PNAS web site). At least twice as many antigens stem from chromosomes 3, 6, 10, 13, and 14 as expected from the overall distribution of the National Center for Biotechnology Information chromosome annotations. Two antigens are located within region 10q24, and three antigens map within 14q21–22. There were no obvious differences between meningioma antigens and randomly selected proteins regarding their subcellular localization, function, association to known cellular pathways, and functional domains.

Discussion

Comprehensive knowledge of the autoantibody repertoire and the frequency of the immune response against a tumor entity are critical in accessing the therapeutic and diagnostic potential of serologically defined tumor antigens. In previous studies, we first reported identification, cloning, and characterization of several immunogenic antigens in meningioma (9–12, 18). Although these studies were largely based on single-serum analysis, we now set out to arrive at a more comprehensive picture of the antibody response in meningioma patients.

We assembled a panel of 62 meningioma-derived antigens, which were tested for reactivity with 48 sera, consisting of 24 sera of meningioma patients and 24 healthy control samples. In total, we found seroreactivity twice as frequent in sera of tumor patients than in control sera. These data confirm our previous results that indicated an elevated antibody response in meningioma patients in comparison with individuals without known disease.

Table 3. Analysis of the combined antibody response against KIAA1344, NKTR, and ANK2

Antibody response against	Expected frequency,* %	Observed frequency, %	Observed/expected frequency
KIAA1344-NKTR	18.8 (4.5/24)	25 (6/24)	1.3
KIAA1344-ANK2	12.5 (3/24)	20.8 (5/24)	1.7
NKTR-ANK2	9.4 (2.3/24)	12.5 (3/24)	1.3
KIAA1344-NKTR-ANK2	4.7 (1.1/24)	12.5 (3/24)	2.7

*$n \times p_1 \times p_2$ ($\times p_3$) with n = number of tested patients (i.e., 24), and p_1, p_2, p_3 = frequency of antibody response (KIAA1344: 12/24; NKTR: 9/24; ANK2: 6/24).

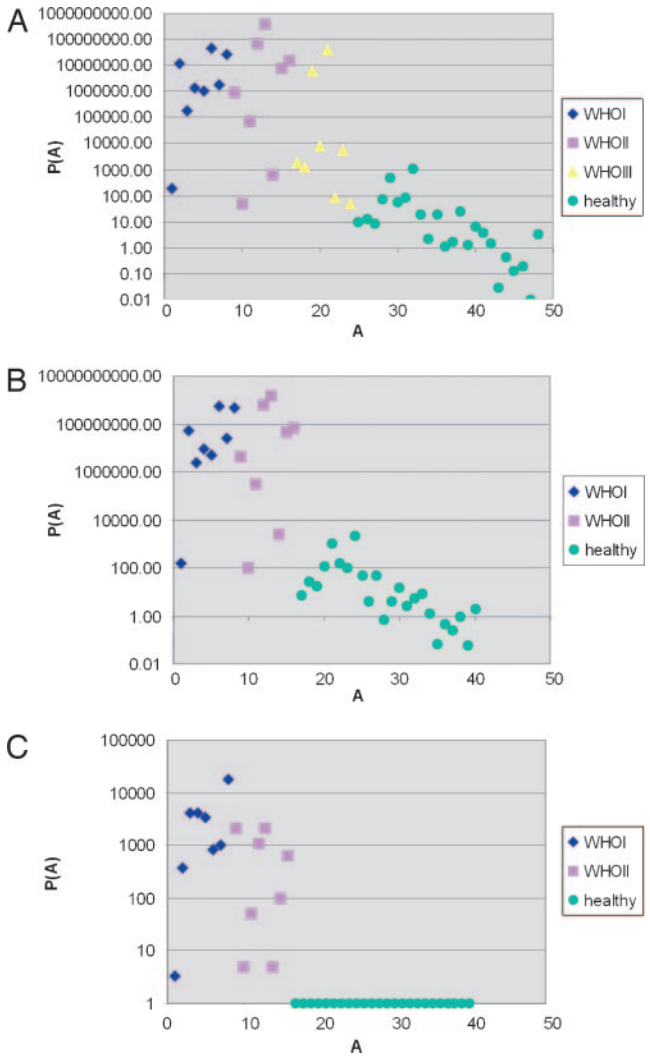

Fig. 4. Separation of meningioma sera and sera of healthy donors by the ''Bayesian approach.'' (*A*) In a first test, we considered all meningioma sera, normal sera, and the complete set of antigens. Each serum is described by an index 1 to 48 (*x* axis). For each serum and its antigen pattern A (described by the set of indices of the antigens 1–62), we computed $P(A)$ (*y* axis). Our approach classifies a serum as meningioma serum if $P(A)$ is larger than a chosen threshold t. A threshold $t = 45$ results in a misclassification rate of 10%, a sensitivity of 1, and a specificity of 0.8. (*B*) In a second test, we considered sera from WHOI and WHOII meningioma, normal sera, and the complete set of antigens. A threshold $t = 90$ results in a misclassification rate of 10%, a sensitivity of 1, and a specificity of 0.8. A threshold at $t = 150$ results in a misclassification rate of 8%, a sensitivity of 0.92, and a specificity of 0.92. (*C*) In a third test, we considered sera from WHOI and WHOII meningioma, normal sera and meningioma-specific antigens only.

MEDICAL SCIENCES

We further compared the antibody response in meningioma of different grades. In general, we found a decline of seroreactivity with increased malignancy. Similar observations were made for other cancer types including colon cancer (14). This decrease of reactivity may be a result of antigen loss as part of a tumor escape mechanism. An interrelation with meningioma patients' treatment is unlikely, because meningioma patients generally undergo anticonvulsant treatment only. Although the mechanisms responsible for the propagation of antigen loss variants are not known, it may be facilitated by epitope immunodominance (19, 20).

As for the specificity of the antibody response, 17 antigens were exclusively detected with sera of meningioma patients, including previously described meningioma-expressed antigens MGEA11 and MGEA5s (10–12). Antigens with the most frequent antibody response in meningioma patients are KIAA1344 protein, NKTR, and SOX2. KIAA1344 encodes a protein of 858 amino acids with a putative thioredoxin 2 domain. The function of KIAA1344 protein is unknown. NKTR is a component of a putative tumor-recognition complex involved in the function of natural killer cells (21) but NKTR also seems to be expressed in a wide range of other tissues. SOX2 is a member of the SOX gene family, which is defined by the SRY-related high mobility group box domain that mediates sequence-specific DNA binding (22) and is involved in the regulation of embryonic development and in the determination of cell fate. SOX2 has been found to be an immunogenic antigen in 41% of small cell lung cancer (SCLC) patients (23). In the latter study, expression of SOX2 was shown for 50% of SCLC cell lines. SOX2 may also be involved in gastric carcinogenesis (24). Notably, another member of the SOX family, SOX6 was recently identified as a glioma antigen (25). KIAA1344 and NKTR were previously not found to react with sera of cancer patients.

Additionally, some of the antigens specifically recognized by meningioma sera were also found in the context of autoimmunity or cancer development. The synaptonemal complex protein SC65 was first characterized as autoantigen in interstitial cystitis (26). TBC1D4 belongs to the TBC domain family that includes TBC1D2 (or PARIS1), which was described as an immunogenic tumor antigen of a prostate cancer cell line (27). C13orf24 is also known as progesterone induced blocking factor 1 (PIBF1). Recently, overexpression of this gene was associated with breast cancer and highly proliferating cells. The PIBF1 protein is associated with the centrosome (28, 29). Notably, loss of progesterone receptor in meningioma patients is correlated with tumor grade and a poor prognosis (30, 31).

Our computational analysis showed that meningioma antigens show a high percentage of both coiled-coil domains and granzyme B cleavage sites with 8 of 37 antigens (21.6%) exhibiting both features. As previously reported, structural features, particularly coiled-coil domains, are probably predominant in systemic autoantigens. More than one-third of the systemic autoantigens contain coiled-coil domains (32), and many autoimmune disease associated antigens are cleaved by granzyme B (33).

Analysis of the chromosomal origin of antigens indicated preferential mapping onto chromosomes and chromosome regions. Recently, a study by Stone *et al.* (34) showed a bias toward antigens encoded on 17q in ovarian tumors. Our results revealed for lung cancer a role of amplified chromosomal domains in the production of autoantibodies (35). Two meningioma antigens stem from chromosome 10q24 and three antigens from 14q21–22. Those chromosome arms are frequently lost in malignant meningioma. These data support the idea that the loss of certain chromosomal regions may contribute to the formation of antigen-loss variants. This idea is also supported by the observed reduction of serum reactivity with malignancy for most of these antigens.

The frequent antibody response against specific antigens offers the potential for new diagnostic targets of meningioma. First, we show that the frequency of the combined occurrence of immunogenic antigens is higher than the expected frequency. Second, we used a computational approach that considers the overall pattern of antigens to identify meningioma patients. Based on our data set, we demonstrate that a statistical learning method allows differentiation between meningioma and normal sera. Any new antigen identified in meningioma sera will further improve the predictive value of the statistical learning method. To be used for clinical purposes, the antigen pattern of course have to be reevaluated in the light of the constantly increasing number of immunogenic antigens, including those that will be deposited in the Cancer Immunome Database.

We greatly acknowledge the technical help of Esther Maldener and Anne Täger and the critical reading of Milena Kitova. This study was supported by Deutsche Krebshilfe Grant 10-1966-Me 4 and by the Center of Bioinformatics in Saarbrücken supported by the Deutsche Forschungsgemeinschaft.

1. Louis, D. N., Scheithauer, B. W., Budka, H., van Deimling, A. & Kepes, J. J. (2000) in *Pathology and Genetics of Tumours of the Nervous System*, eds. Kleihues, P. & Cavenee, W. K., (Int. Agency Res. Cancer, Lyon, France) 176–189.
2. Meese, E., Blin, N. & Zang, K. D. (1987) *Hum. Genet.* **77,** 349–351.
3. Dumanski, J. P., Rouleau, G. A., Nordenskjold, M. & Collins, V. P. (1990) *Cancer Res.* **50,** 5863–5867.
4. Simon, M., von Deimling, A., Larson, J. J., Wellenreuther, R., Kaskel, P., Waha, A., Warnick, R. E., Tew, J. M., Jr., & Menon, A. G. (1995) *Cancer Res.* **55,** 4696–4701.
5. Weber, R.G., Bostrom, J., Wolter, M., Baudis, M., Collins, V. P., Reifenberger, G. & Lichter, P. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14719–14724.
6. Zang, K. D. (2001) *Cytogenet. Cell Genet.* **93,** 207–220.
7. Ruttledge, M. H., Sarrazin, J., Rangaratnam, S., Phelan, C. M., Twist, E., Merel, P., Delattre, O., Thomas, G., Nordenskjold, M., Collins, V. P., *et al.* (1994) *Nat. Genet.* **6,** 180–184.
8. Wellenreuther, R., Kraus, J. A., Lenartz, D., Menon, A. G., Schramm, J., Louis, D. N., Ramesh, V., Gusella, J. F., Wiestler, O. D. & von Deimling, A. (1995) *Am. J. Pathol.* **146,** 827–832.
9. Heckel, D., Brass, N., Fischer, U., Blin, N., Steudel, I., Tureci, O., Fackler, O., Zang, K. D. & Meese, E. (1997) *Hum. Mol. Genet.* **6,** 2031–2041.
10. Heckel, D., Comtesse, N., Brass, N., Blin, N., Zang, K. D. & Meese, E. (1998) *Hum. Mol. Genet.* **7,** 1859–1872.
11. Comtesse, N., Maldener, E. & Meese, E. (2001) *Biochem. Biophys. Res. Commun.* **283,** 634–640.
12. Comtesse, N., Niedermayer, I., Glass, B., Heckel, D., Maldener, E., Nastainczyk, W., Feiden, W. & Meese, E. (2002) *Oncogene* **21,** 239–247.
13. Sahin, U., Tureci, O., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I. & Pfreundschuh, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 11810–11813.
14. Scanlan, M. J., Gout, I., Gordon, C. M., Williamson, B., Stockert, E., Gure, A. O., Jager, D., Chen, Y. T., Mackay, A., O'Hare, M. J. & Old, L. J. (2001) *Cancer Immun.* **1,** 4–21.
15. Hastie, T., Tibshirani, R. & Friedman, J. (2001) in *Elements of Statistical Learning* (Springer, New York).
16. Backes, C., Kuentzer, J., Lenhof, H.-P, Comtesse, N. & Meese, E. (2005) *Nucleic Acids Res.* **33,** in press.
17. Thornberry, N. A., Rano, T. A., Peterson, E. P., Rasper, D. M., Timkey, T., Garcia-Calvo, M., Houtzager, V. M., Nordstrom, P. A., Roy, S., Vaillancourt, J. P., *et al.* (1997) *J. Biol. Chem.* **272,** 17907–17911.
18. Comtesse, N., Heckel, D., Racz, A., Brass, N., Glass, B. & Meese, E. (1999) *Clin. Cancer Res.* **5,** 3560–3568.
19. Schreiber, H., Wu, T. H., Nachman, J. & Kast, W. M. (2002) *Semin. Cancer Biol.* **12,** 25–31.
20. Khong, H. T. & Restifo, N. P. (2002) *Nat. Immunol.* **3,** 999–1005.
21. Anderson, S. K., Gallinger, S., Roder, J., Frey, J., Young, H. A. & Ortaldo, J. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 542–546.
22. Wegner, M. (1999) *Nucleic Acids Res.* **27,** 1409–1420.
23. Gure, A. O., Stockert, E., Scanlan, M. J., Keresztes, R. S., Jager, D., Altorki, N. K., Old, L. J. & Chen, Y.-T. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 4198–4203.
24. Li, X. L., Eishi, Y., Bai, Y. Q., Sakai, H., Akiyama, Y., Tani, M., Takizawa, T., Koike, M. & Yuasa, Y. (2004) *Int. J. Oncol.* **24,** 257–263.
25. Ueda, R., Iizuka, Y., Yoshida, K., Kawase, T., Kawakami, Y. & Toda, M. (2004) *Oncogene* **23,** 1420–1427.
26. Ochs, R. L., Stein, T. W., Jr., Chan, E. K., Ruutu, M. & Tan, E. M. (1996) *Mol. Biol. Cell* **7,** 1015–1024.
27. Zhou, Y., Toth, M., Hamman, M. S., Monahan, S. J., Lodge, P. A., Boynton, A. L. & Salgaller, M. L. (2002) *Biochem. Biophys. Res. Commun.* **290,** 830–838.
28. Polgar, B., Kispal, G., Lachmann, M., Paar, C., Nagy, E., Csere, P., Miko, E., Szereday, L., Varga, P. & Szekeres-Bartho, J. (2003) *J. Immunol.* **171,** 5956–5963.
29. Lachmann, M., Gelbmann, D., Kalman, E., Polgar, B., Buschle, M., Von Gabain, A., Szekeres-Bartho, J. & Nagy, E. (2004) *Int. J. Cancer* **112,** 51–60.
30. Hsu, D. W., Efird, J. T. & Hedley-Whyte, E. T. (1997) *J. Neurosurg.* **86,** 113–120.
31. Perry, A., Cai, D. X., Scheithauer, B. W., Swanson, P. E., Lohse, C. M., Newsham, I. F., Weaver, A. & Gutmann, D. H. (2000) *J. Neuropathol. Exp. Neurol.* **59,** 872–879.
32. Dohlman, J. G., Lupas, A. & Carson, M. (1993) *Biochem. Biophys. Res. Commun.* **195,** 686–696.
33. Casciola-Rosen, L., Andrade, F., Ulanet, D., Wong, W. B. & Rosen, A. (1999) *J. Exp. Med.* **190,** 815–826.
34. Stone, B., Schummer, M., Paley, P. J., Thompson, L., Stewart, J., Ford, M., Crawford, M., Urban, N., O'Briant, K. & Nelson, B. H. (2003) *Int. J. Cancer* **104,** 73–84.
35. Brass, N., Racz, A., Bauer, C., Heckel, D., Sybrecht, G. & Meese, E. (1999) *Blood* **93,** 2158–2166.