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Improving Seroreactivity-Based Detection of Glioma[1]

Nicole Ludwig*, Andreas Keller†, Sabrina Heisel*, Petra Leidinger*, Veronika Klein*, Stefanie Rheinheimer*, Claudia U. Andres†, Bernhard Stephan‡, Wolf-Ingo Steudel§, Norbert M. Graf¶, Bernhard Burgeth#, Joachim Weickert#, Hans-Peter Lenhof†,2 and Eckart Meese*,2

*Department of Human Genetics, Medical School, Saarland University, Building 60, 66421 Homburg/Saar, Germany; †Center for Bioinformatics, Saarland University, Building E.1.1, 66041 Saarbrücken, Germany; ‡Department of Haematology and Transfusion Medicine, Medical School, Saarland University, Building 75, 66421 Homburg/Saar, Germany; §Department of Neurosurgery, Medical School, Saarland University, Building 90, 66421 Homburg/Saar, Germany; ¶Department of Paediatric Oncology and Haematology, Medical School, Saarland University, Building 9, 66421 Homburg/Saar, Germany; #Mathematical Image Analysis Group, Saarland University, 66041 Saarbrücken, Germany

Abstract

Seroreactivity profiling emerges as valuable technique for minimal invasive cancer detection. Recently, we provided first evidence for the applicability of serum profiling of glioma using a limited number of immunogenic antigens. Here, we screened 57 glioma and 60 healthy sera for autoantibodies against 1827 *Escherichia coli* expressed clones, including 509 in-frame peptide sequences. By a linear support vector machine approach, we calculated mean specificity, sensitivity, and accuracy of 100 repetitive classifications. We were able to differentiate glioma sera from sera of the healthy controls with a specificity of 90.28%, a sensitivity of 87.31% and an accuracy of 88.84%. We were also able to differentiate World Health Organization grade IV glioma sera from healthy sera with a specificity of 98.45%, a sensitivity of 80.93%, and an accuracy of 92.88%. To rank the antigens according to their information content, we computed the area under the receiver operator characteristic curve value for each clone. Altogether, we found 46 immunogenic clones including 16 in-frame clones that were informative for the classification of glioma sera *versus* healthy sera. For the separation of glioblastoma *versus* healthy sera, we found 91 informative clones including 26 in-frame clones. The best-suited in-frame clone for the classification glioma sera *versus* healthy sera corresponded to the vimentin gene (*VIM*) that was previously associated with glioma. In the future, autoantibody signatures in glioma not only may prove useful for diagnosis but also offer the prospect for a personalized immune-based therapy.

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Introduction

Gliomas are the most common primary brain tumor with an annual incidence of approximately 6 in 100,000 persons [1]. Gliomas arise from the neuroepithelial tissue with the main morphologic subtype of gliomas being astrocytomas representing 75% of all gliomas, followed by oligodendrogliomas with 8.4%. The World Health Organization

Address all correspondence to: Nicole Ludwig, Kirrberger Strasse, Bldg. 60, 66421 Homburg, Germany. E-mail: n.ludwig@mx.uni-saarland.de
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[2]The authors have contributed equally as senior authors.
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(WHO) classifies astrocytomas into four malignancy grades [2]. WHO grade I or pilocytic astrocytomas comprise 5.8% of all gliomas and are the most frequent brain tumors in children and adolescents. WHO II or diffuse astrocytomas account for 1.5% of all gliomas and are characterized by tendencies to recur and to progress to higher-grade astrocytomas. WHO III or anaplastic astrocytomas that comprise 7.5% of gliomas frequently progress after a mean interval of 2 years. Glioblastoma multiforme (WHO grade IV) that account for 18.5% of all primary brain tumors are fast-growing, highly infiltrating tumors with a mean survival of approximately 1 year after diagnosis.

Treatment of malignant glioma includes maximal surgery followed by radiotherapy with concomitant and adjuvant temozolomide therapy. Although recent therapeutic advances have improved standard treatment only few patients experience prolonged survival. New targeted therapies include infection with a p53-producing adenovirus to reestablish normal p53 expression in gliomas and lyses of glioma cells by modified herpes simplex virus. Besides various attempts to targeted therapy for glioma [3], there are also attempts to use the immune response for therapeutical purposes including vaccination with an EGFRvIII peptide or autologous dendritic cells pulsed with glioma lysate (reviewed in Selznick et al. [4]). One major challenge for glioma treatment is the tremendous heterogeneity especially of glioblastoma. An approach to address this challenge is the identification of a large number of tumor antigens that can be used both for therapy and for diagnosis. Lately, immunogenic antigens have been combined to define autoantibody signatures that have been reported for a variety of cancers including prostate, lung, ovarian, and brain cancers [5–19]. Recently, we identified 35 antigens that allowed detecting a specific autoantibody signature of glioma [12]. Glioma sera were separated form control sera with a specificity of 86.1%, a sensitivity of 85.2%, and an accuracy of 85.8%. Here, we set out to largely extend the number of antigens that cause an immune response in patients whose conditions were diagnosed as glioma. In detail, we screened an array encompassing 1827 clones for reactivity against serum autoantibodies of glioma patients by using a newly developed image analysis software. We also ask how specific the new antigens are for gliomas.

Materials and Methods

Patients' Sera

Blood samples of glioma patients and healthy controls were obtained from the Departments of Neurosurgery and Hemastaseology of Saarland University with patients' informed consent. Serum was isolated from serum gel monovettes by centrifugation and stored at −70°C. Table 1 provides more detailed information on age and sex of the patients and control group.

Screening Procedure

We screened high-density protein macroarrays arraying 38,016 *Escherichia coli* expressed proteins of the hex1 library [20] with 150 sera of various cancer and non–cancer sera including 30 sera of glioma patients of all four WHO grades. All clones that were positive in at least one serum were selected and spotted in duplicates on subarray filters. These subarrays encompassing 1827 immunoreactive *E. coli* clones were then screened with 57 sera of glioma patients and 60 sera of healthy controls. In brief, filters were washed twice with TBSTT (TBS, 0.05% Tween 20, and 0.5% Triton X-100) and four times with TBS and then blocked 2 hours in blocking solution (TBS, 0.05% Tween 20, and 3% dry milk). Subsequently, membranes were incubated overnight with serum dilution (1:1000 in TBST/3% dry milk). Serum was then stored at 4°C for a second round of incubation. Membranes were washed three times with TBST and then incubated in stripping solution at 70°C. Filters were subsequently washed two times in TBST and four times in TBS and then again blocked for 2 hours with a blocking solution. Membranes were incubated with the stored serum dilution overnight. Arrays were then washed three times with TBST. Detection of bound autoantibodies was carried out by incubation with secondary antibody (1:1000 rabbit anti–human immunoglobulins G and A and immunoglobulin M–Cy5 [H + L; Dianova, Hamburg, Germany] in blocking solution). Finally, filters were washed four times in TBST, two times in TBS and subsequently dried overnight. Signals were detected by scanning with Typhoon 9410 scanner (GE Healthcare, Uppsala, Sweden).

Table 1. Mean Age and Sex of the Considered Patient and Control Groups.

	WHO		*n*	Mean Age	Range
Glioma	All grades	All	57	44.3	4-78
		Male	36	46.6	13-78
		Female	21	40.4	4-75
	WHO II/III	All	22	39.5	16-71
		Male	11	43.1	16-71
		Female	11	35.8	22-53
	WHO IV	All	28	52.7	15-78
		Male	21	50.8	15-78
		Female	7	58.3	35-75
Healthy		All	60	38.2	20-61
		Male	41	38.2	20-61
		Female	19	38.2	22-53

Image Analysis and Statistics

Spot intensity was computed by a novel computer-aided image analysis procedure. In brief, macroarray was divided in target areas that contained all pixels of a single protein spot. Subsequently, the target area was clustered in foreground and background pixels. By applying the so-called black top-hat operator known from the image analysis field, the dark foreground spots were extracted from the pale background. Finally, mean intensity of all foreground pixels was calculated, and the average over the intensities of both replicates for each clone was computed.

We carried out standard quantile normalization to minimize array-to-array variations. Because our image analysis method also detects not available spots, we were able to exclude spots that show more than 10 not-available spots on all arrays. The remaining 1417 clones were used for the classifications of glioma sera *versus* healthy sera, WHO IV glioma sera *versus* healthy sera and WHO IV glioma sera *versus* WHO II/III sera by a linear support vector machine. Altogether, 100 repetitions of a standard 10-fold cross-validation were performed, and mean sensitivity, specificity, and accuracy for the three classification tasks was calculated. To test for overtraining, we performed 100 classification runs with randomly permuted class labels.

As a measure of the information content of single antigens for their ability to differentiate glioma sera *versus* healthy sera, WHO IV glioma sera *versus* healthy sera, and WHO IV glioma sera *versus* WHO II/III sera, the area under the receiver operator characteristic curve (ROC) value (AUC) was computed. The ROC curve is specificity as function of 1 − sensitivity. For each antigen, all normalized intensity values in glioma and healthy sera were used as thresholds to discriminate glioma sera from the healthy controls. For all these thresholds, glioma sera with an intensity value above the threshold were considered as true-positive (TP) and glioma sera with an intensity value below the threshold were

Table 2. Mean Values of Specificity, Sensitivity, and Accuracy for the Classifications of Glioma *versus* Healthy Controls, WHO IV Glioma *versus* Healthy Controls, and WHO IV Glioma *versus* WHO II/III Glioma.

Classification	Specificity	Sensitivity	Accuracy
Glioma *vs* healthy	90.28 (89.90-90.67)	87.31 (86.82-87.81)	88.84 (88.50-89.17)
Random	51.03 (49.82-52.25)	47.47 (46.06-48.88)	49.30 (48.14-50.45)
WHO IV glioma *vs* healthy	98.45 (98.22-98.68)	80.93 (80.48-81.37)	92.88 (92.66-93.09)
Random	73.52 (72.40-74.64)	23.96 (22.19-25.73)	57.75 (56.67-58.83)
WHO IV glioma *vs* WHO II/III glioma	42.86 (42.06-43.67)	57.00 (56.01-57.99)	50.78 (50.13-51.43)
Random	41.36 (39.19-43.54)	56.79 (55.24-58.33)	50.00 (48.45-51.55)

Values were calculated in 100 cross-validated classification runs. As control, 100 classification runs were done with randomly permutated class labels. CIs are given in parentheses.

considered as false-negative (FN). Accordingly, healthy sera with intensity value below the threshold were considered as true-negative (TN) and healthy sera with intensity value above the threshold were considered as false-positive (FP). Subsequently, sensitivity [TP / (TP + FN)] and specificity [TN / (TN + FP)] of all thresholds were used to calculate ROC curve and AUC value of the considered antigen. If intensity values of the considered antigen in glioma sera are generally higher than in healthy sera, AUC values range from 0 to 0.5. AUC values ranging from 0.5 to 1 confer to a higher mean intensity of the antigen in healthy sera compared with glioma sera. We considered antigens with AUC values below 0.3 or above 0.7 as informative for the given classification. Finally, we computed the frequency of seroreactivity against the antigens in the considered groups by using a threshold of 50 for positive seroreactivity.

Results

Classification of Glioma Sera Using Protein Macroarray Screening

We screened 57 glioma and 60 healthy sera for autoantibodies against 1827 *E. coli* expressed clones, including 509 in-frame peptide sequences. Signal intensity was measured by a novel automated procedure as detailed in the Materials and Methods section. After quantile normalization, 410 clones were excluded from further considerations because these spots were labeled not available in more than 10 screened sera by the image analysis procedure. By a linear support vector machine approach, we discriminated glioma sera *versus* healthy sera, WHO IV glioma *versus* healthy sera, and WHO IV glioma sera *versus* WHO II/III glioma sera and calculated mean specificity, sensitivity, and accuracy of 100 repetitive classifications (Table 2). Thereby we could differentiate glioma sera from sera of the healthy control group with a specificity of 90.28% (95% confidence interval [CI], 89.90%-90.67%), a sensitivity of 87.31% (95% CI, 86.82%-87.81%), and an accuracy of 88.84% (95% CI, 88.50%-89.17%). We were also able to differentiate WHO IV glioma sera from healthy sera with a specificity of 98.45% (95% CI, 98.22%-98.68%), a sensitivity of 80.93% (95% CI, 80.48%-81.37%), and an accuracy of 92.88% (95% CI, 92.66%-93.09%). The separation of WHO IV glioma sera from WHO II/III glioma sera only yielded a specificity of 42.86% (95% CI, 42.06%-43.67%), a sensitivity of 57.00% (95% CI, 56.01%-57.99%), and an accuracy of 50.78% (95% CI, 50.13%-51.43%), which means that, with this set of tumor-associated antigens, a separation of these two groups is not possible. Likewise, separation of WHO II glioma *versus* healthy, WHO III glioma *versus* healthy, and WHO IV glioma *versus* WHO II glioma was not possible (data not shown). As controls, we performed a random, stratified permutation of class labels for the all classifications. In this control, we obtained mean results for specificity, sensitivity, and accuracy of approximately 50%.

Information Content of Antigens

To rank the antigens according to their information content for the four classification tasks, we computed the AUC value for each clone as detailed in the Materials and Methods section. Table 3 provides an overview on the distribution of AUC values among the clones. Most antigens provided no or little information for the classification tasks: only 3% to 7% of the clones yielded AUC values of above 0.7 or below 0.3, which represents the AUC range in that an antigen is considered informative. Altogether, 46 clones including 16 in-frame clones were considered informative for the classification of glioma sera *versus* healthy sera. For the separation of WHO IV glioma *versus* healthy sera, 91 clones including 26 in-frame clones were considered informative. Table 4 lists these in-frame clones with their corresponding AUC values. The best-suited in-frame clone for the classification glioma sera *versus* healthy sera corresponded to the vimentin gene (*VIM*) with an AUC of 0.225 (Figure 1). Interestingly, the same clone was also best suited for the classification of WHO IV glioma sera *versus* healthy sera

Table 3. Distribution of Clones According to the AUC Values That Were Determined for the Classification of Glioma *versus* Healthy Controls and WHO IV Glioma *versus* Healthy Controls.

AUC Value	Glioma *vs* Healthy		WHO IV Glioma *vs* Healthy		WHO IV Glioma vs WHO II/III Glioma	
	No. All Clones	No. In-frame Clones	No. All Clones	No. In-frame Clones	No. All Clones	No. In-frame Clones
0.0-0.1	0	0	0	0	0	0
0.1-0.2	0	0	4	**3**	0	0
0.2-0.3	38	**16**	55	**22**	2	0
0.3-0.4	219	69	218	71	125	41
0.4-0.5	513	166	455	149	509	155
0.5-0.6	506	140	452	113	555	143
0.6-0.7	133	0	201	32	216	51
0.7-0.8	8	0	30	**1**	10	**1**
0.8-0.9	0	0	2	0	0	0

Number of all clones and number of in-frame clones is given for each AUC interval. In-frame clones with AUC values lower than 0.3 or above 0.7 were considered informative for a classification task (bold).

Table 4. AUC Value of Highly Informative Antigens for the Classification of Glioma Sera *versus* Healthy Controls and of WHO IV Glioma Sera *versus* Healthy Controls.

Clone ID	Ensemble ID	Gene Name	AUC Glioma *vs* Healthy	AUC WHO IV Glioma *vs* Healthy	Known Antigen
B17512	ENSG00000026025	*VIM*	**0.225**	**0.173**	+
M19590	ENSG00000140988	*RPS2*	**0.226**	**0.188**	+
K19595	ENSG00000142937	*RPS8*	**0.232**	**0.175**	
J15577	ENSG00000026025	*VIM*	**0.247**	**0.261**	+
F16565	ENSG00000083812	*ZNF324*	**0.248**	**0.275**	
G19583	ENSG00000122406	*RPL5*	**0.251**	**0.261**	
H18530	ENSG00000091140	*DLD*	**0.258**	**0.263**	
E14506	ENSG00000198708	*LOC388720*	**0.265**	**0.236**	
G20580	ENSG00000105220	*GPI*	**0.266**	**0.255**	
G13583	ENSG00000167588	*GPD1*	**0.282**	**0.235**	
D14506	ENSG00000198708	*LOC388720*	**0.287**	**0.279**	
O03589	ENSG00000140988	*RPS2*	**0.289**	**0.242**	+
C09514	ENSG00000170759	*KIF5B*	**0.291**	**0.289**	
H22512	ENSG00000125817	*CENPB*	**0.292**	**0.288**	+
H23512	ENSG00000180626	*ZNF594*	**0.293**	**0.282**	+
F10567	ENSG00000005194	*CIAPIN1*	**0.293**	**0.285**	
K19538	ENSG00000182670	*TTC3*	0.304	**0.254**	+
I17585	ENSG00000127903	*FLJ12955*	0.314	**0.266**	
D18552	ENSG00000153317	*DDEF1*	0.306	**0.279**	
O11577	ENSG00000105894	*PTN*	0.308	**0.279**	
H21512	ENSG00000131095	*GFAP*	0.325	**0.277**	+
F12523	ENSG00000075340	*ADD2*	0.349	**0.279**	
M18573	ENSG00000111653	*ING4*	0.309	**0.288**	
O20573	ENSG00000151914	*DST*	0.316	**0.292**	+
M15565	ENSG00000166313	*APBB1*	0.332	**0.294**	
B09544	ENSG00000106244	*PDAP1*	0.664	**0.714**	+

+ indicates that antigen is deposited in the Cancer Immunome Database.
* Numbers in bold indicate informative antigens.

with an AUC value of 0.173. Altogether, 16 clones were informative for both the classification of glioma sera *versus* healthy sera and of WHO IV glioma sera *versus* healthy sera. These clones include dihydrolipoyl dehydrogenase (*DLD*), centromere protein B (*CENPB*), and 40S ribosomal protein S2 (*RPS2*). Other informative clones for the discrimination of WHO IV glioma sera *versus* healthy include glial fibrillary acidic protein (*GFAP*), inhibitor of growth family member 4 (*ING4*), and pleiotrophin (*PTN*).

Frequency of Seroreactivity against the Antigens

To assess a frequency of seroreactivity for the examined antigens, we considered a serum as positive for autoantibodies against an antigen if the intensity value of the corresponding spot is above 50. Subsequently, the frequency of seroreactivity against every antigen after the normalization procedure was computed as mean reactivity in glioma and healthy controls. Table 5 shows the distribution of the remaining 1417 antigenic clones including the 391 in-frame clones in the given frequency intervals. There are 23 clones including 4 in-frame clones reacting with more than 60% of glioma sera. Forty-three clones including 7 in-frame clones reacted with more than 60% of healthy sera. Because many antigens react with high frequencies in glioma and healthy sera, we computed the number of antigens reacting twice as frequent with glioma sera than with healthy sera. Thereby we obtained 47 clones including 7 in-frame clones that react at least twice as frequent with glioma sera than with healthy sera and, furthermore, that react with at least 20% of glioma sera (Table 6). The clone with the highest frequency in glioma represented the gene nonhomologous end-joining factor 1 (*NHEJ1*) reacting with 36.8% of glioma sera but also with 18.3% of healthy sera. Notably, two of the clones, namely tetratricopeptide repeat protein 3 (*TTC3*) and amyloid β A_4 precursor protein-binding family B member 1 (*APBB1*), react at least twice as often with glioma sera than with healthy sera and, furthermore, provide good information for the discrimination of WHO IV glioma sera *versus* healthy sera. *TTC3* provided an AUC value of 0.254 for the discrimination and was reactive with 26.3% of glioma sera and 6.7% of healthy sera. *APBB1* yielded an AUC value of 0.294 and reacted with 21.1% of glioma sera and 10.0% of healthy sera.

Discussion

The usefulness of autoantibody signatures for the detection of cancer has been shown by our group and others for multiple cancer types including meningioma, prostate, lung, and ovarian cancers [5–19]. We could demonstrate a complex seroreactivity pattern in meningioma, and by using this pattern, we could differentiate meningioma from healthy controls with a specificity of 96.2%, a sensitivity of 84.5%, and an accuracy of 90.3% [10,11]. Wang et al. [5] separated sera of prostate cancer patients from sera of a control group with a specificity of 88.2% and a sensitivity of 81.6%. Zhong et al. [6] were able to discriminate sera of patients with non–small cell lung cancer from normal sera with a specificity of 95% and a sensitivity of 90%. A study on lung adenocarcinoma patients yielded a specificity of 86% and a sensitivity of 85% [7]. Chatterjee et al. [8] could differentiate ovarian cancer with an average specificity of 90% and a sensitivity of 32%, whereas Erkanli et al. [9] classified sera of epithelial ovarian cancer patients with an AUC value of 0.86 without noticing sensitivity or specificity.

Although the knowledge about a humoral antitumor immune reaction in glioma in general is several decades old, the more detailed view on the involved antigens and the concept of using this response to diagnose glioma evolved over the last decade [21–23]. After the development of new methods to identify tumor-associated antigens in greater number, such as SEREX or T7 phage display, the number of glioma-associated antigens also grew [24,25]. Until now, 61 glioma-associated antigens are listed in the Cancer Immunome Database [26], and several more are published by our group and others [12,27–29]. After identifying a set of glioma-associated antigens, we were the first to screen a large number of glioma sera and various controls including

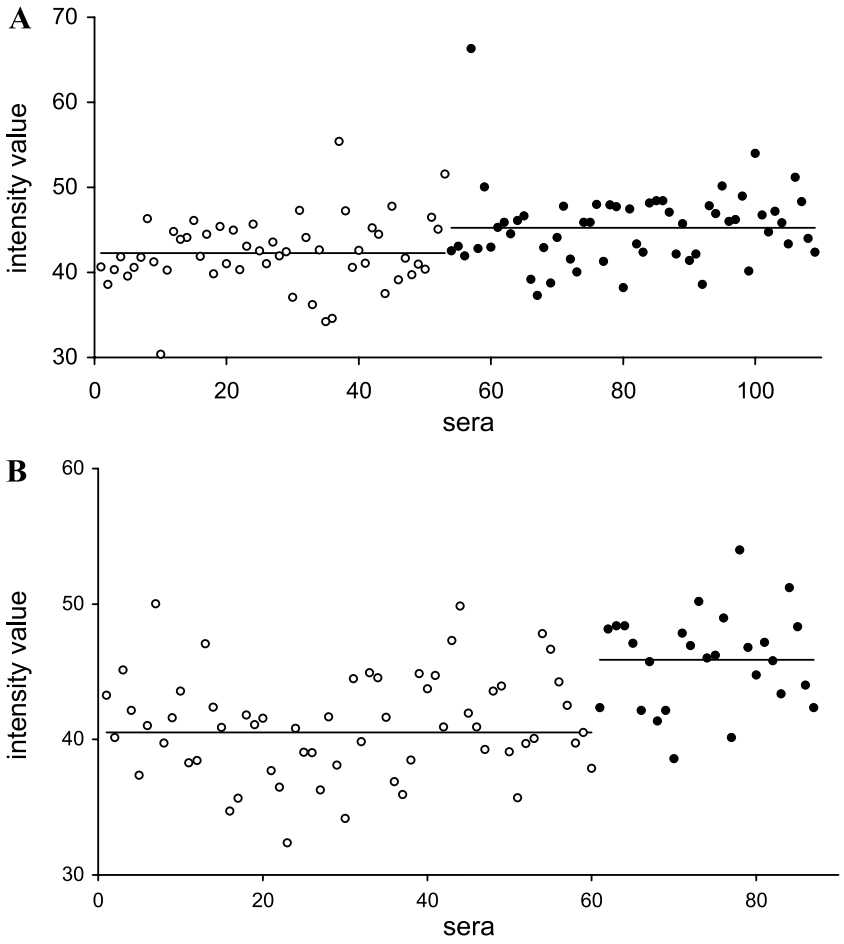

Figure 1. Seroreactivity against the clone representing VIM. The graphs show the intensity values of seroreactivity (*y*-axis) in the tested sera (*x*-axis). Mean seroreactivity value is indicated as horizontal line. (A) Intensity values of seroreactivity against clone *VIM* are provided for healthy controls (empty circle) and for glioma sera (full circle). (B) Intensity values of seroreactivity against clone *VIM* are provided for healthy controls (empty circle) and WHO IV glioma sera (full circle).

other tumor and nontumor pathologic lesions of the brain and to use the detected seroreactivity patterns for glioma differentiation [12]. By using a SEREX-derived method, we were able to discriminate glioma sera from healthy controls with a specificity of 87.8% and sensitivity of 85.2% and an accuracy of 86.5%, and furthermore, we could differentiate glioma sera from controls with other pathologic lesions of the brain yielding a specificity of 86.1% and a sensitivity of 85.2% and an accuracy of 85.8% [12].

The two major goals of the present study were to identify novel immunogenic antigens in glioma and to test their value for the differentiation of glioma patients from healthy controls. To achieve these aims, we screened a novel array encompassing 1827 disease-related immunogenic clones with sera of glioma patients and healthy controls and evaluated the results with state of the art statistical learning methods. Thereby we were able to discriminate glioma sera from healthy controls with a specificity of 90.28%, a sensitivity of 87.31%, and an accuracy of

Table 5. Distribution of Clones According to the Seroreactivity Frequency Determined for Glioma and for Healthy Controls (First and Second Column).

Frequency Range	Glioma		Healthy		Glioma (Twice)	
	No. All Clones	No. In-frame Clones	No. All Clones	No. In-frame Clones	No. All Clones	No. In-frame Clones
0%	51	17	176	62	—	—
0-10%	536	176	552	169	152	91
10-20%	444	117	305	78	109	49
20-30%	187	40	163	36	34	5
30-40%	100	20	93	22	7	2
40-50%	54	14	55	8	3	0
50-60%	22	3	30	9	2	0
60-70%	16	4	27	5	1	0
70-80%	4	0	11	2	0	0
80-90%	3	0	4	0	0	0
90-100%	0	0	1	0	0	0

The third column lists clones that react at least twice as often with glioma sera than with healthy control sera. Number of all clones and number of in-frame clones are given for each frequency interval.

Table 6. Immunogenic Clones Frequently Detected with Glioma Sera.

Clone ID	Ensemble ID	Gene Name	Frequency Glioma	Frequency Healthy	Known Antigen
K09567	ENSG00000187736	*NHEJ1*	36.84	18.33	
M24570	ENSG00000004534	*RBM6*	33.93	16.67	
C07557	ENSG00000197907		26.32	11.67	
G11565	ENSG00000101460	*MAP1LC3A*	26.32	11.67	
K19538	ENSG00000182670	*TTC3*	26.32	6.67	+
B14594	ENSG00000114942	*EEF1B2*	22.81	8.47	
M15565	ENSG00000166313	*APBB1*	21.05	10.00	

+ indicates that antigen is deposited in the Cancer Immunome Database.

88.84%. In comparison to our SEREX-derived classifier, we could improve specificity and accuracy of the classification of glioma sera *versus* healthy controls by using this extended set of immunogenic clones [12]. Furthermore, we could even differentiate WHO IV glioma sera from healthy sera with a specificity of 98.45%, a sensitivity of 80.93%, and an accuracy of 92.88%.

To assess the value of single antigens for a classification task, we ranked the antigens according to their AUC value. Antigens best suited to differentiate glioma sera or WHO IV glioma sera from healthy controls showed AUC values of 0 to 0.3 or 0.7 to 1. This led to the identification of several novel antigens previously not associated with glioma as well as antigens already known in context of glioma. *GFAP*, *ING4*, *PTN*, and *TTC3* offered a high discrimination power for the classification of WHO IV glioma sera *versus* healthy sera. *GFAP* and *TTC3* have been deposited at the Cancer Immunome Database as autoantigens detected with glioma serum [20]. *ING4* has already been identified as a glioma-associated antigen in our previous study that used the classic SEREX approach [12]. *GFAP* has been found to be strongly expressed in WHO IV glioma [30]. The level of expression of *PTN* was associated with histopathologic grade in astrocytoma [31].

The intermediate filament protein vimentin (VIM) was best suited for the classification of glioma sera *versus* healthy sera and of glioblastoma sera *versus* healthy sera. In normal brain, VIM expression is specific for cells of glial origin. Accordingly, VIM is strongly expressed in glioma especially in glioblastoma and is associated with enhanced motility and invasive growth in astrocytoma [30,32]. Furthermore, VIM is expressed on the surface of apoptotic cells and has been shown to induce the production of autoantibodies [33,34]. Autoantibodies against VIM have previously been identified in pancreatic cancer and rheumatoid arthritis [35,36]. Although VIM is strongly expressed in glioma, its expression in normal glial cells remains a major obstacle for the development of a vimentin-based therapy approach.

To achieve a curative treatment of glioma, future clinical settings will be focused on two aspects: the removal of the primary tumor and the deletion of the diffusely distributed glioma cells in the surrounding brain parenchyma. Whereas elimination of the primary tumor mass will include conventional radiotherapy and resection, elimination of single tumor cells within the brain will include conventional chemotherapy and newly developed immune-based therapy strategies as gene therapy and vaccination. Although there are many clinical trails regarding treatment of malignant glioma or glioblastoma, most of them are focused on different combinations of chemotherapeutic agents with radiation therapy and resection. Only a few immune therapeutic strategies have reached phase 1 or 2 clinical trails. These include vaccination of the patients with tumor lysate–pulsed, autologous dendritic cells or vaccination with tumor-isolated proteins or with synthetic peptides targeting epidermal growth factor receptor variant III [37–39]. Although some of these studies show promising results, for further improvement of immune therapy–based treatment in glioma patients, there is a need for identification of more target proteins. Autoantibody signatures generated from an extended set of autoantigens in glioma can prove useful for glioma therapy in two ways, namely early diagnosis and personalized therapy. Although there is no evidence that early diagnosis yields to an improved clinical outcome in patients with malignant glioma, this evidence was found for several other cancers such as prostate or colon cancer [40,41]. It has also been shown that autoantibodies can be detected years before onset of the disease, for example, against the tumor protein p53 in lung cancer [42]. In addition, glioma-associated antigens identified by the autoantibody signatures may offer themselves as possible future targets for an immune-based therapy in glioma. The determination of autoantibody signatures for each patient and the identification of specific targets may contribute to a personalized medicine for glioma patients.

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