Hyperphosphorylated paratarg-7: a new molecularly defined risk factor for monoclonal gammopathy of undetermined significance of the IgM type (IgM-MGUS) and Waldenstrom's macroglobulinemia

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Abstract

We recently described paratarg-7 (P-7), a protein of unknown function, as the target of 15% of IgA and IgG paraproteins in MGUS and multiple myeloma (MM). To determine the frequency of paratarg-7 as a paraprotein target in IgM-MGUS and Waldenstrom’s macroglobulinemia (WM), sera from patients with IgM-MGUS/WM were tested for reactivity with recombinant paratarg-7 by ELISA. The specificity of the paraprotein-mediated reaction was demonstrated by absorption studies and cloning of the respective B-cell receptor. The paraproteins of 18 (9 WM and 9 IgM-MGUS) of 161 (11%) patients reacted with paratarg-7. Isoelectric focusing and phosphatase treatment revealed that paratarg-7 was hyperphosphorylated (pP-7) in all patients with an anti-paratarg-7 specific IgM paraproteins tested. Since only 4/200 (2%) of healthy controls were carriers of pP-7, pP-7 carrier state is associated with a significantly increased risk (odds ratio= 6.2; p=0.001) for developing IgM-MGUS/MW. Family analyses revealed that the pP-7 carrier state is inherited as a dominant trait. After IgA/IgG-MGUS and multiple myeloma, IgM-MGUS/WM is the second neoplasia associated with pP-7 carrier state. The dominant inheritance of pP-7 explains cases of familial IgM-MGUS/WM and enables the identification of family members at increased risk.

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Introduction

Waldenström’s macroglobulinemia (WM) is classified as an IgM secreting B-cell non-Hodgkin lymphoma characterized primarily by lymphoplasmacytic infiltrates in the bone marrow accompanied by hypersecretion of monoclonal immunoglobulin M (IgM). It corresponds to the lymphoplasmacytic lymphoma (LPL) as defined by the World Health Organisation classification. Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic precursor condition commonly preceding MM and IgM-MGUS may precede development of WM.

Although age, race, sex, and pre-existing IgM-MGUS are recognized risk factors, the etiology of WM is largely unknown. Several studies demonstrated a significantly increased risk for WM after infections with hepatitis B virus, immunodeficiency virus and rickettsiosis and found an increased risk of WM among individuals with a personal history of autoimmune disease.

Genetic factors play an important role, with 20% of patients demonstrating a familial predisposition. Several studies of multiple affected families have been published showing familial clustering of LPL and WM. A recent study showed that family members of MGUS and LPL-WM patients have a significant higher risk for MGUS/LPL-WM. Environmental influences, chance occurrence, and inherited factors may all contribute to familial clusters. The evidence of somatic immunoglobulin gene mutations in WM indicates a role for antigenic stimulation in the development of WM. A causal relationship between MGUS/WM and chronic antigenic stimulation has been suggested by the results of several studies. Because of the rarity of LPL-WM, only a few studies have assessed the role of various types of chronic antigenic stimulatory conditions in relation to risk of developing MGUS/WM, and their results have been conflicting, hence the identification of the antigenic stimuli of B-cell neoplasms might be of considerable importance.
In a systematic study covering a broad spectrum of potential antigens using a modified SEREX (serological identification of antigens by expression cloning) approach, which allows for the systematic screening of putative antibody-antigen interactions, even if neither the antigen nor the antibody is known. In a complementary approach using a human fetal brain-derived macroarray and IgA or IgG paraprotein-containing sera, the paraproteins of 29/192 patients (15.1%) consecutive MGUS and MM patients reacted with paratarg-7. Paratarg-7 is identical to STOML2 (stomatin [EPB72]-like), also known as HSPC108 or stomatin-like-protein and SLP-2, a protein of unknown function, which is expressed in all human tissues. In an extension of our earlier study, the high frequency of paratarg-7 specific paraproteins in the sera of MGUS/MM patients (35/252; 14%) was confirmed in a subsequent study. Moreover, it was shown that all patients with paratarg-7 specific paraproteins were carriers of a hyperphosphorylated version of the protein (pP-7) and that the carrier state of pP-7 is inherited in a dominant fashion. Since only 2% of healthy Germans are carriers of pP-7, pP-7 carrier state is associated with an increased risk (odds ratio: 7.9) to develop IgA/IgG-MGUS/MM. Thus, pP-7 is the first molecularly defined inherited risk factor known for any hematological neoplasm to date. Because of the autosomal-dominant inheritance of pP-7, we set out to determine the prevalence of pP-7 carrier state and the frequency of pP-7 specific paraproteins in other malignancies.

Methods

Patients and controls
This study was approved by the local ethical review board (“Ethikkommission der Ärztekammer des Saarlandes”) and conducted according to the Declaration of Helsinki. Between January 2005 and February 2009 serum samples from 67 consecutive patients treated at Saarland University...
Medical School, 44 patients from the Bing Center for Waldenstrom's Macroglobulinemia, Dana Farber Cancer Institute, Harvard Medical School (Boston, MA) and 50 patients from the University of Athens School of Medicine, Department of Clinical Therapeutics (Athens, Greece) were included in this study in which serum protein electrophoresis had identified a monoclonal spike which was demonstrated to contain a monoclonal IgM paraprotein by immunofixation. The control group consisted of 200 healthy employees of Saarland University Medical School. “Healthy” was defined as having no monoclonal immunoglobulin by serum electrophoresis and immunofixation and being healthy as diagnosed by the Medical Officer of Saarland University in the pre-donation check-up. Whenever possible, human materials were obtained during routine diagnostic or therapeutic procedures and stored at -80°C until use. Written informed consent was obtained from patients, their relatives and controls for studying paratarg-7 in lysates of their whole peripheral blood.

**Family analyses**

All relatives, identified by pedigree analysis, were contacted through the patients. Human materials were obtained from 25 relatives of 4 families from patients with IgM-MGUS/WM (2 families with MGUS and 2 families with Waldenström´s macroglobulinemia).

**Isoelectric focusing (IEF)**

Blood samples were centrifuged and washed with PBS followed by lysis in lysis buffer containing 8 M urea, 0.1 M NaH2PO4, 0.01 M Tris-HCL and 0.1% NP40 (15 min, 20°C) and stored at -20°C until use. Equal volumes of sample and loading buffer were mixed. Samples were analysed by isoelectric focusing on a gel with a fixed pH gradient (pH 3-10) according to the
manufacturer`s instructions (Novex® pH 3-10, Invitrogen, Germany, Karlsruhe) followed by an immunoblot screening.

**Immunoblot screening**

After lysates from whole peripheral blood were separated by isoelectric focusing (IEF) or SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), the proteins were transferred to a Immobilon-P PVDF (polyvinylidene difluoride) membrane (Millipore Immobilon, Eschborn, Germany) by semi-dry blotting. The membrane was blocked overnight at 4°C in TBST/milk buffer (10% (v/v) milk in 10 mM Tris/HCl, pH 7.5, 150 mM NaCl and 0.1% (v/v) Tween 20), washed and incubated for 1 h at room temperature with serum in TBST (paraprotein-containing serum from patients at a dilution of 1:10^8 and from controls at a dilution of 1:10^3). After three washings in TBST, the membranes were incubated for 1 h at room temperature (RT) with mouse anti-human IgG–POX antibody (BioRad, California, USA) diluted 1:3000 in TBST, subsequently washed in TBST and followed by detection using Pharmacia’s ECL (enhanced chemiluminescence) system (General Electrics, Connecticut, USA).

**Phosphatase treatment of paratarg-7**

Blood samples were centrifuged and washed with PBS followed by lysis in LS buffer (10 mM TrisHCL pH8, 30 min, 4°C). After increasing the concentration of TrisHCl to 100 mM, alkaline phosphatase was added (1U/µl per 500 µl lysate) and incubated at 37°C overnight. The phosphatase was inactivated by heating at 80°C for 10 min. Equal volumes of sample and loading buffer were mixed, followed by IEF and immunodetection as described above.
**Paratarg-7 ELISA**

The paratarg-7 ELISA using full-length recombinant paratarg-7 was performed as described by us previously 20. For testing the paratarg-7-specificity of the recombinant BCR, the paratarg-7 ELISA was performed according to standard protocols using the expressed and purified recombinant Fab antibodies.

**Absorption studies**

Recombinant His6-tagged paratarg-7 protein or total blood lysate was immobilized on HiTRAP-chelating columns (Pharmacia, Freiburg, Germany) containing 1 ml nickel-nitrilotriaceticacid-agarose (Qiagen, Hilden, Germany) following a published procedure (www.flemingtonlab.com). Patients’ serum (100 µl) was diluted 1:2 (v/v) in PBS and depleted by passing 3 times over the paratarg-7 column. The flow-through was checked by immunofixation and serum protein electrophoresis.

**Cloning and expression of B-cell receptors**

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) lymph node or bone marrow biopsies according to the manufacturer’s instructions (QIAamp DNA FFPE Tissue-Kit, Qiagen, Germany, Hilden). DNA was subjected to a semi-nested PCR for VH and VK/VL genes. The protocol and primers used for the amplifications were described previously 21. Products were analyzed by electrophoresis on agarose gels and sequenced. Sequencing results were analyzed online using IMGT/V-QUEST 22 on IMGT®, the international ImMunoGeneTics information system® (http://www.imgt.org)23.
Results

Carrier state of hyperphosphorylated paratarg-7 in hematological neoplasias and solid tumors

Since pP-7 is inherited as a dominant trait and is expressed in all cells of pP-7 carriers, we looked for the prevalence of the pP-7 carrier state in various hematological neoplasias and solid tumors (Tab. 1). There was no signal in any of the tumor types investigated for an increased prevalence of pP-7 carriers except for IgM-MGUS and WM.

Paratarg7 as the target of IgM paraproteins

Serum samples from 161 consecutive patients with MGUS and WM in Germany (n=67), USA (n=44) and Greece (n=50) with a monoclonal spike in the serum electrophoresis which was confirmed by immunofixation to be a monoclonal IgM paraprotein were included in this study. There was no difference with respect to age, gender (all patients), progression, M-protein level and stage of the disease between patients with a paratarg-7 specific paraprotein and patients having a non-paratarg-7-specific paraprotein. The sera were tested at a dilution of 1:10^8 for anti-paratarg-7 reactivity using a paratarg-7 ELISA. The paraproteins of 9/51 IgM-MGUS patients (17.6%) and of 9/110 (8.2%) WM patients reacted specifically with paratarg-7, resulting in an overall rate of 18/161 or 11.2% (Tab. 2), proving paratarg-7 as a paraprotein target in a significant proportion of patients with IgM-MGUS/WM. The anti-paratarg-7 reactivity of these sera had titers ranging from 1:10^8 to 1:10^{10}. None of the sera from healthy controls reacted at a dilution of ≤1:10^2. Lower serum dilutions were not tested because they cause too much background in the paratarg-7 ELISA.
The specificity of the paraprotein-mediated reaction was demonstrated by absorption studies with recombinant paratarg-7 and by cloning the B-cell receptor. For the absorption study recombinant His6-tagged paratarg-7 protein was immobilized on a Ni\(^{2+}\) agarose. After three passages of the anti-paratarg-7 positive IgM paraprotein-containing sera over the paratarg-7 column, the monoclonal spike in the electrophoresis of the flow-through and the clonal band in the immunofixation had disappeared, while this was not the case with a serum containing an IgM paraprotein with a non-paratarg-7 reactivity (Fig. 1). There was no relevant overall difference in the serum levels of the monoclonal IgM paraproteins when the 9 pP-7 MGUS and 9 pP-7 WM patients were compared with the corresponding pP-7 negative patients. For cloning and expression of the B-cell receptor DNA was isolated from bone marrow cells and paraffin-embedded lymph nodes, respectively, from three patients with an anti-paratarg-7 specific paraprotein and 5 BCR with non-p-7 specificity as controls. The immunoglobulin genes (V\(\text{H}\), V\(\text{K}\), V\(\lambda\)) were characterized by PCR and cloned into a phagemid vector in order to produce Fab fragments. The Fabs were characterized by protein gel electrophoresis and Western blot and their specificity was demonstrated by an ELISA with recombinant paratarg-7 as a coat (Figure 2).

**Analysis of paratarg-7**

Sequence analyses of paratarg-7 from all patients analyzed (n=6) were identical, excluding mutations or SNPs as the reason for the autoimmunogenicity of paratrag-7 in the respective patients. However, 2D-gel electrophoresis, isoelectric focusing and phosphatase treatment revealed that paratarg-7 was hyperphosphorylated in all patients with an anti-paratarg-7 specific IgM paraprotein. To date all patients with a P-7 specific paraproteins (>50 IgA/IgG paraproteins and 14 IgM paraproteins), from whom peripheral blood cells were available were shown to be carriers of pP-7. Conversely, no pP-7 carrier was found among more than >500 patients with a
paraprotein of specificity other than pP-7. Of the patients included in this study, peripheral blood cells were not available from the 4 Boston patients with P-7 specific paraproteins. All other patients with P-7 specific paraproteins were shown to be carriers of pP-7. In contrast, only 4 of 200 (2.0%) healthy blood donors were carriers of hyperphosphorylated paratarg-7 (Tab. 2). Thus, carriers of hyperphosphorylated paratarg-7 have a significantly increased risk (odds ratio= 6.2; 95%-CI: 2.0-18.6; p=0.001) for developing IgM-MGUS/MW (Tab. 2).

**Inheritance of hyperphosphorylated paratarg-7**

The analysis of relatives of IgM-MGUS/WM patients with an anti-paratarg-7 specific paraprotein revealed that the hyperphosphorylated state of this protein is inherited as a dominant trait (Figs. 3 & 4). The members of 4 families with patients who had a paratarg-7 specific IgM paraprotein in their serum gave written consent to be studied for hyperphosphorylated paratarg-7 carrier state. The pedigree in figure 3A shows the family of a 50-year old man with WM (II.1). He had a paratarg-7-specific IgM paraprotein (titre: 1:10⁹) and was a carrier of hyperphosphorylated paratarg-7. His mother (I.1) and his son (III.1) did not have a paraprotein or an anti-paratarg-7 reactivity in their serum at a dilution of 1:10², but isoelectric focusing revealed that they were also carriers of the hyperphosphorylated paratarg-7 protein. Moreover, in the family shown here, a male-to-male transmission (from II-1 to III-1) was observed, allowing to state the mode of inheritance more precisely as autosomal-dominant. An uncle of the patient (I.2) and his daughter (II.5) were also carriers of hyperphosphorylated partarg-7, but they did not have a partarg-7-specific paraprotein. In all four families tested, the analysis of relatives of MGUS/WM patients with an anti-paratarg-7 specific paraprotein confirmed that the hyperphosphorylated state of paratarg-7 is inherited in a dominant fashion.
Discussion

In this study 18/161 (11.2%) paraproteins from IgM-MGUS/WM (9 from patients with Waldenstrom's macroglobulinemia and 9 from patients with IgM-MGUS) reacted specifically with paratarg-7 (Tab. 2), proving paratarg-7 as the first antigen identified as a paraprotein target in a significant proportion of patients with IgM-MGUS/WM. The specificity of the paraprotein-mediated reaction was demonstrated by absorption studies with recombinant paratarg-7 and by cloning the B-cell receptor from involved tissues of patients with a paratarg-7 specific paraprotein. Sequence analyses of paratarg-7 from all patients analyzed (n=6) were identical, excluding mutations or SNPs as the reason for the autoimmunogenicity of paratarg-7 in the respective patients. Moreover, IEF and phosphatase treatment showed that all analyzed patients with paratarg-7 specific IgM paraproteins were carriers of a hyperphosphorylated version of the protein (pP-7). Since only 2.0% of healthy controls are carriers of pP-7, pP-7 carrier state is associated with a significantly increased risk to develop IgM MGUS/WM.

Although it is well established that family members of WM patients face an increased risk for developing WM and related B-cell malignancies 7, the genetic basis of enhanced susceptibility in these families remains undefined. Family analyses of relatives of IgM MGUS/WM patients with an anti-paratarg-7 specific paraprotein showed that the hyperphosphorylated state of this protein is inherited as a dominant trait. There have been a number of studies of familial aggregation of WM, implicating both environmental and inherited factors 4-7;24. Several reports suggest that gene mutations or genetic polymorphisms may be associated with the risk of WM 4;6;13. However, results have been inconsistent and significant findings have not been replicated convincingly. Hyperphosphorylated paratarg-7 is the first molecularly characterized structure that provides a plausible explanation for the familial clustering of cases of IgM MGUS/WM, at least in cases with a paratarg-7 specific paraprotein as
shown for one of our families analyzed in this study (Fig. 1). The index patient, a 50-year old man was a carrier of hyperphosphorylated paratarg-7 and had a paraprotein with specificity for this antigenic target. His mother and one of his uncles were also carriers of pP7, but they did not have a paraprotein or an anti-paratarg-7 reactivity in their serum at a dilution of 1:10^2. Moreover, in the family shown here, a male-to-male transmission (from II-1 to III-1) was observed, allowing for describing the mode of inheritance more precisely as autosomal-dominant. It is now possible to investigate whether previously reported cases of familial WM could also be explained by the carrier state of hyperphosphorylated paratarg-7.

The frequency of the carrier state of hyperphosphorylated paratarg-7 among patients with IgM-MGUS/WM and in healthy controls reveals a 6.2 times increased risk to develop MGUS/WM for carriers of the hyperphosphorylated protein. This is, to the best of our knowledge, the highest odds ratio of a single risk factor reported to date for IgM-MGUS/WM. Since the pP-7 carrier state is inherited as a dominant trait and expressed life-long, age does not affect the prevalence of the pP-7 carrier state. According to the hypothesis of chronic antigenic stimulation, increasing age would be associated with prolonged antigenic stimulation and thus an increased incidence of pP-7 associated MGUS and WM, respectively. Indeed, the youngest patient with a pP-7 associated MGUS/WM in this study was 37 years old. Median age was 67.8 years (range 37 to 90 years). There was no difference with respect to age, gender (all patients), M-protein level, stage of the disease between patients with an anti-paratarg-7 specific paraprotein and the rest. Since the control group (median age 40 years) was 25 years younger than the patients with paraproteins (60 years), we can not exclude that the 4/200 healthy pP-7 carriers will develop an anti-paratarg specific paraprotein on longer follow-up. The same applies to family members with the hyperphosphorylated paratarg-7 carrier state. If this were the case, however, it would further increase the odds ratio of pP-7 carriers to develop IgM-MGUS-WM.
The results obtained in IgM-MGUS/WM are similar to recent observations made in 252 patients with IgG- or IgA-MGUS/MM where hyperphosphorylated paratarg-7 was also associated with a significantly increased risk of developing IgG- and IgA-MGUS/MM (odds ratio: 7.9; p<0.001) and was also shown to be inherited in a dominant fashion \(^9\). There was no indication that pP-7 carrier state is associated with any hematological neoplasia or solid tumor other than MGUS, MM and WM (Tab. 1), and it is intriguing that pP-7 is associated with two B-cell neoplasms which present with quite a different biology and very different clinical findings. This suggests that B-cells with anti-paratarg-7 specificity are prone to malignant transformation during different stages of their maturation.

In contrast to the carrier state of pP-7, which is under exclusive genetic control, the nature of the immune response against pP-7 is complex and might involve both genetic and environmental factors. The frequency of pP-7 as an antigenic target and/or stimulus for paraprotein-producing clones and the availability of many families with MGUS/WM and MM patients with the pP-7 carrier state now allow for the analysis of tumor-host interactions in the presence and absence of the antigen in the respective patients and family members, and to study more specifically the role of environmental factors and immunoregulatory deficiencies, such as the recently reported dysfunction of regulatory T cells \(^26\) in patients with MGUS and multiple myeloma. Finally, the fact that hyperphosphorylated paratarg-7 functions as the antigenic target of the paraproteins of all MGUS/WM patients with hyperphosphorylated paratarg-7, suggests that the hyperphosphorylated protein plays a role in the development of IgM-MGUS/WM. The hyperphosphorylation of paratarg-7 appears to be the most obvious and likely reason for its autoimmunogenicity. Indeed, it has been shown that „phosphoepitopes“ have a higher binding affinity to the MHC complex and induce stronger CD8\(^+\) \(^27\) and CD4\(^+\) T-cell responses \(^28\). Whether hyperphosphorylated paratarg-7 induces the development of MGUS/WM by chronic antigenic
stimulation or whether it is only a marker or an epiphenomenon of another dominantly inherited susceptibility to develop MGUS/WM can now be investigated in the respective patients and their (not yet) affected relatives.

Acknowledgements

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Authorship

SG designed the experiments and wrote the manuscript; K-D P designed the experiments; AW performed the experiments and analyzed the results. ET and MD recruited and analyzed Greece patients for the study. ST and ZH recruited and analyzed Boston patients for the study. MZ did the statistical analysis. NF and ER performed and analysed the experiments. DN and YY collected and tested the patients with neoplasias other than MGUS/MM/WM and solid tumors. MP designed the study and wrote the manuscript.

Financial disclosures

KDP and MP have applied for a relevant patent. Otherwise, none of the authors has a conflict of interest.
References


Table 1: Carrier state of hyperphosphorylated paratarg-7 in patients with neoplasias of different origins.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Carrier State</th>
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<tbody>
<tr>
<td>Follicular Lymphoma</td>
<td>1/50</td>
</tr>
<tr>
<td>MCL</td>
<td>1/29</td>
</tr>
<tr>
<td>DLBCL</td>
<td>1/100</td>
</tr>
<tr>
<td>M. Hodgkin</td>
<td>1/58</td>
</tr>
<tr>
<td>CLL</td>
<td>0/50</td>
</tr>
<tr>
<td>ALL/AML</td>
<td>1/33</td>
</tr>
<tr>
<td>Breast</td>
<td>1/50</td>
</tr>
<tr>
<td>Lung</td>
<td>0/50</td>
</tr>
<tr>
<td>Colon</td>
<td>0/50</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>1/50</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1/66</td>
</tr>
</tbody>
</table>

Total: 8/586
Table 2. Prevalence of hyperphosphorylated paratarg-7 carrier state in patients with IgM-MGUS/WM and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>IgM-MGUS/WM</th>
<th>healthy controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>hyperphosphorylated</td>
<td>18 (11.2%)</td>
<td>4 (2.0%)</td>
<td>22</td>
</tr>
<tr>
<td>paratarg-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wild-type paratarg-7</td>
<td>143 (88.8%)</td>
<td>196 (98.0%)</td>
<td>339</td>
</tr>
<tr>
<td>total</td>
<td>161</td>
<td>200</td>
<td>361</td>
</tr>
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Odds Ratio: 6.2; 95% CI: 2.0 -18.6; p =0.001
Figure Legends

Figure 1. Absorption of IgM paraproteins with recombinant paratarg-7. (a): IgM paraprotein with paratarg-7 reactivity and absorption with a paratarg-7 column. (b): IgM paraprotein with nonparatarg-7 reactivity and absorption by a partarag-7 column (control). Upper panels: serum electrophoresis before and after absorption, lower panels: immunofixation before and after absorption with paratarg-7. The absorption studies of 3 sera containing a paratarg-7 reactive IgM paraprotein and 2 IgM control paraproteins with paratarg-7 non-reactivity yielded identical results.

Figure 2. Reactivity and specificity of expressed paratarg-7 specific B-cell receptors. For this ELISA recombinant paratarg-7 was used as a coat. The B-cell receptor of a patient with a paratarg-7 specific IgM paraprotein which was cloned from the patient’s lymph node involved by Waldenstrom’s macroglobulinemia (black circles) had the same anti-praraterg-7 specificity as the patient’s serum containing the IgM paraprotein (black squares). There was no reactivity in the serum of a patient with an IgM-MGUS with a non-paratarg-7 specific paraprotein (grey rhombus). The B-cell receptor of a patient with chronic lymphocytic leukemia with specificity for the FAM32A protein showed no reactivity (control, black triangle). The starting dilution of the sera was 10e8 and the starting concentration of the expressed B-cell receptors was 10 µg/ml.

Figure 3. Pedigree of a WM patient with a paratarg-7 specific IgM paraprotein carrying the hyperphosphorylated paratarg-7. A: The pedigree shows the family of a 50-year old man with WM (II.1), having a paratarg-7 reactive paraprotein and carrying the hyperphosphorylated state of this protein. The pattern of a hyperphosphorylated paratarg-7 carrier state in this family is consistent with an autosomal dominant trait. B: Immunostaining of lysate bands derived from
whole peripheral blood lysates from family members carrying wild-type (I.3; I.4; II.2; II.3; II.4; III.2; III.3; III.4; III.5; III.6) and hyperphosphorylated paratarg-7 (I.1; I.2; II.1; II.5; III.1) after IEF. The numbers indicate family members in different generations.

**Figure 4. Pedigree of a MGUS patient with a paratarg-7 specific IgM paraprotein carrying the hyperphosphorylated paratarg-7.** A: The pedigree shows the family of a 77 year old man with IgM-MGUS (I.1), having a paratarg-7 reactive paraprotein and carrying the hyperphosphorylated state of this protein. B: Immunostaining of lysate bands derived from whole peripheral blood lysates from family members carrying wild-type (I.2; III.1) and hyperphosphorylated paratarg-7 (I.1; II.1; III.2) after IEF. The numbers indicate family members in different generations.
Figure 1

- **before depletion**
  - a
  - b

- **after depletion**
  - a
  - b
Figure 3

A

I

II

III

B

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