DOI: 10.1111/j.1365-2362.2012.02679.x

ORIGINAL ARTICLE

# Overexpression of G protein-coupled receptor 5D in the bone marrow is associated with poor prognosis in patients with multiple myeloma

Johanna Atamaniuk*, Andreas Gleiss†, Edit Porpaczy*, Birgit Kainz*, Thomas W. Grunt‡, Markus Raderer‡, Bernadette Hilgarth*, Johannes Drach‡, Heinz Ludwig§, Heinz Gisslinger*, Ulrich Jaeger*, and Alexander Gaiger*

*Department of Medicine I, Division of Hematology and Hemostaseology, Medical University Vienna, Austria, †Department of Medical Statistics and Informatics, Medical University Vienna, Austria, ‡Department of Medicine I, Division of Oncology, Medical University Vienna, Austria, §Department of Internal Medicine I, Division of Hematology and Oncology, Wilhelminen-Hospital, Vienna, Austria

## ABSTRACT

**Background** G protein-coupled receptor 5D (GPRC5D) is a novel surface receptor. As this new subtype of G protein-coupled receptors was discovered, little is known about the role of this gene.

**Materials and methods** In this retrospective study, we investigated GPRC5D mRNA expression by real-time polymerase chain reaction (RT-PCR) in bone marrow (BM) of 48 patients with multiple myeloma (MM).

**Results** Highly variable levels of GPRC5D (median, 288; quartiles, 17–928) were detected in patients with MM, whereas only low expression was detected in normal tissues (median, 1; quartiles, 1–23). High mRNA expression of GPRC5D correlated positively with high plasma cell count in bone marrow ($r$ = 0·64, $P$ < 0·001), high $\beta_2$-microglobulin ($r$ = 0·42, $P$ = 0·003) and poor-risk cytogenetics: deletion 13q14 (rb-1), $P$ = 0·003; and 14q32 translocation t(4;14)(p16;q32), $P$ = 0·029. GPRC5D mRNA expression showed a significant correlation with overall survival ($P$ = 0·031). The estimated overall survival of patients expressing GPRC5D above or below the median of 288 was 43·9% vs. 70·2% at 48 months. Here, we report, for the first time, the association of GPRC5D expression and cancer.

**Conclusions** Overexpression in poor-risk myeloma, low expression in normal tissues and cell surface expression identify GPRC5D as a potential novel cancer antigen. Our data demonstrate that GPRC5D is a prognostic factor in MM correlating with other major risk factors.

**Keywords** Bone marrow, cytogenetics, GPRC5D mRNA expression, multiple myeloma.

Eur J Clin Invest 2012; 42 (9): 953–960

## Introduction

Recent advances in the treatment of multiple myeloma (MM) resulted in higher response rates and improved progression free and overall survival [1–4]. Nevertheless, MM remains an incurable disease in the vast majority of patients, and there is a high unmet medical need for new therapeutic options [5–7]. The number of clinical trials, and thus the ability to test new drugs either as a single agent or in combinations with other compounds, is hampered by the relatively low incidence of MM. Therefore, one of the challenging issues in myeloma is the decision which compound or combination of drugs to move into clinical trials. *In vitro* testing of novel agents and drug combinations is becoming of increasing importance for the selection of those compounds for clinical studies [7–10]. Novel markers expressed in myeloma cells, associated with clinical features such as overall survival, the international staging system (ISS) and cytogenetics, might serve as surrogate markers in preclinical testing or even serve as potential therapeutic targets [11,12].

Recently, a new subtype of human orphan family C G protein-coupled receptors GPRC5D has been identified [13]. Orphan receptors do not possess a recognized ligand. RAIG1, 2, 3 (retinoic acid-induced gene one, two, three) and GPRC5D

together represent the C5 subfamily of GPRCs. GPRC5D has seven transmembrane segments and is expressed in cell membranes [13]. The GPRC5D gene that is mapped on chromosome 12p13.3 contains three exons and spans about 9·6 kb. The large first exon encodes the seven-transmembrane domain [13]. High and medium levels of mRNA expression were found in kidney, pancreas, small intestine, spleen and testis, whereas low expression was detected in lung, colon, prostate, thymus and leucocytes [13]. GPRC5D is also expressed in differentiating cells that produce hard keratin [14]. In mice, predominant expression of mouse GPRC5D in adult skin with little expression in either embryonic or adult lungs and unremarkable expression in other adult tissues were reported [15]. Little is known about the biochemistry of these receptors, their ligands and consecutive downstream signalling events [13–15].

Thus far, there are no data on the role of GPRC5D in cancer. Here, we report, for the first time, the expression of GPRC5D in the bone marrow of patients with MM. Association of mRNA expression with the plasma cell count in the bone marrow, β2- microglobulin plasma levels, ISS, cytogenetics [deletion 13q14 and t(4;14)] and OS established GPRC5D as a novel prognostic marker in MM. The ability to monitor GPRC5D mRNA expression semi-quantitatively by real-time PCR indicates its potential in preclinical testing. Overexpression in poor-risk myeloma, low expression in normal tissues, the possible presence of T-helper and CTL epitopes and cell membrane expression identify GPRC5D as a potential novel cancer antigen.

## Design and methods

Bone marrow samples of 48 patients with MM were included in this retrospective study, after informed consent was obtained. The study was approved by our ethics committee IRB (Internal Review Board) number MUW 11/2005. The median age was 59·8 years (range at date of diagnosis from 36 to 94 years; in quartiles, 55·2–69·8). According to the International staging system for MM [16], 47% of the patients had stage I, 21% stage II and 32% stage III.

Patients were treated at the Medical University of Vienna or at Wilhelminen Hospital, Vienna with various regimens or sequences thereof including melphalan, dexamethason, VAD, bortezomib and/or autologous bone marrow transplantation (six of 48).

RNA was isolated from unsorted frozen bone marrow cells (BM MNCs). For controls, mRNA was isolated from 10 samples of bone marrow originating from healthy donors. In addition, mRNA from 10 samples of mononuclear cells (MNC) from peripheral blood of healthy volunteers, tissues and cell lines was isolated. The following cell lines and tissues were tested: K-562 (myeloid cell line), Ramos (B cell line), DAUDI (B cell line), CCRF (T cell line), Jurkat (T cell line), REH (T ALL cell line) and RL7 (B cell line); testis, heart, spinal cord, foetal liver, foetal brain, placenta, thymus, salivary gland, thyroidal gland, adrenal gland, trachea, lung, liver, kidney, muscle, spleen, prostate, tonsil, brain (whole), brain cerebellum, uterus and bone marrow [RNA was obtained from BD Biosciences Clontech (Palo Alto, CA, USA)].

### Interphase fluorescence *in situ* hybridisation

Relevant genetic aberrations were assessed by fluorescence *in situ* hybridisation (FISH). FISH studies were performed with probes specific for 13q14 (rb-1) to detect a deletion 13[del 13(13q14)] and for the $C_H/V_H$ regions of the immunoglobulin heavy chain gene locus at 14q32 to detect any 14q-translocation [t(14q)]. In patients with evidence for a t(14q), translocation-specific probe sets to detect a t(11;14)(q13;q32) or t(4;14)(p16;q32) were used. All probes were obtained from Vysis Inc. (Downers Grove, IL, USA) and used at concentrations according to the manufacturer's recommendations. In every patient, at least 100 clonal plasma cells were examined for each abnormality under an immunofluorescence microscope equipped with a triple band-pass filter to simultaneously visualize 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI), fluorescein isothiocyanate (FITC)/Spectrum-green and Spectrum-orange (Axioplan 2; Zeiss, Jena, Germany).

### Real-time PCR

RNA was isolated from unsorted frozen bone marrow (BM) from the time of diagnosis ($n$ = 48), using RNA-Bee™ (Tel-Test Inc., Friendswood, TX, USA). For cDNA synthesis, first-strand cDNA synthesis kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) was used. Real-time PCR was performed with the ABI Prism 7000 Sequence Detector (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. β-actin (VIC™-labelled predeveloped TaqMan® Assay reagent; Applied Biosystems) was used as an endogenous control. PCR was carried out in a 25 μL reaction volume using 1 μL of a 1 : 5 cDNA dilution with *GPRC5D*-specific primers (Assays-on-Demand Gene Expression system; Applied Biosystems; Assay ID, Hs 00218660-R1), probes and TaqMan® Universal Master Mix without AmpErase®UNG. All samples were run in duplicates. The *GPRC5D* mRNA expression levels given in this study represent the mean value of these two independent measurements. Real-time PCR negativity was defined by the absence of amplified product after 40 cycles. As a control, *GPRC5D* mRNA expression levels were measured in 10 BM and 10 peripheral blood (PB) samples derived from healthy donors (ranged from 0·11 to 6·0). Normal peripheral blood MNC was used as a calibrator (defined to be 1), and *GPRC5D* mRNA expression in patient specimens was compared to these normal PB samples in 48 patients. To exclude contamination, negative controls (water) were run with each experiment. Delta values

 

were calculated as the difference between CT (threshold cycle) values of β-actin and *GPRC5D* ($\Delta CT = CT_{\beta\text{-actin}} - CT_{GPRC5D}$).

### Statistical analysis

Levels of GPRC5D, age and other continuous variables are presented as median and quartiles owing to their skewed distribution. The association between GPRC5D and continuous variables is quantified using Spearman's correlation coefficient. To compare GPRC5D between levels of categorical variables, Wilcoxon's rank-sum test or Kruskal–Wallis test was used, respectively.

To graphically represent the potential influence of GPRC5D on overall survival, GPRC5D was dichotomized at the sample median. Kaplan–Meier estimates are plotted and accompanied with *P*-values from logrank tests. Univariate and multi-variable proportional hazards Cox models were used to investigate the influence of GPRC5D on survival more thoroughly. Hazard ratios with 95% confidence intervals and *P*-values are given. In these models, GPRC5D and β2-Microglobulin were log-transformed using binary logarithms, and thus hazard ratios refer to the effect of doubling the respective variable. Median follow-up time was estimated using the inverse Kaplan–Meier method [17].

The reported *P*-values are the results of two-sided tests. *P*-values ≤ 0·05 were considered to be statistically significant. All computations have been performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA, 2008). Reporting of the study is compliant with STROBE statement (along with references) and the broader EQUATOR guidelines [18].

## Results

### Expression of GPRC5D mRNA in multiple myeloma

Levels of GPRC5D distributed over four orders of magnitude were detected in the bone marrow of patients with MM (median, 288; IQ, 17–928) (Fig. 1). This indicated that GPRC5D might be used as a prognostic marker. Table 1 shows the association of GPRC5D expression with patient's characteristics and prognostic factors. Significant correlation was observed between expression of GPRC5D and bone marrow plasma cell infiltration, β2-microglobulin as well as cytogenetics. Median plasma cell infiltration was 28·5%.

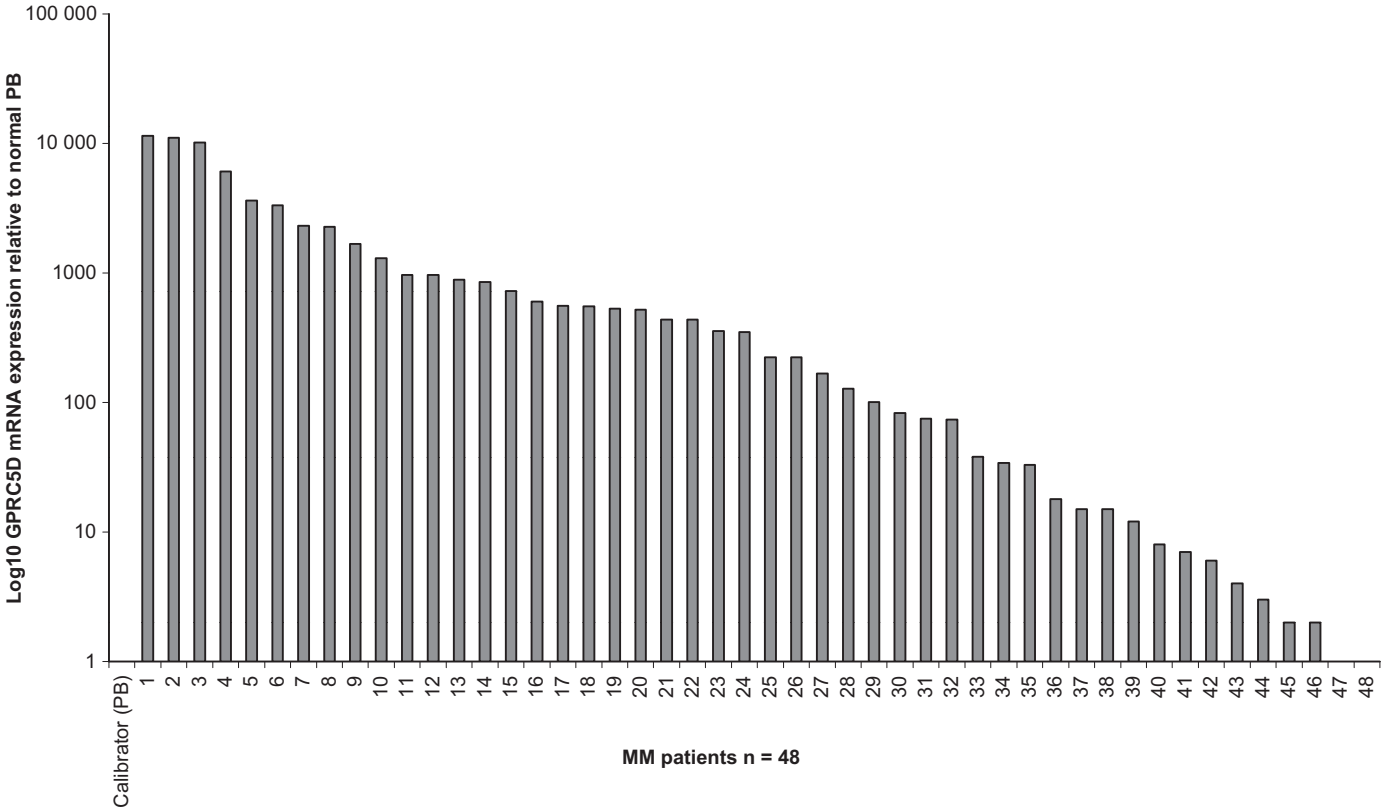


**Figure 1** Levels of GPRC5D over four orders of magnitude were detected in the bone marrow of 48 patients with multiple myeloma. Normal peripheral blood MNC was used as a calibrator, and GPRC5D mRNA expression in patient specimens was compared to these normal PB samples in 48 patients.

**Table 1** Patient characteristics and association of GPRC5D with important variables

| | No. of patients | GPRC5D median (quartiles) | Correlation with GPRC5D* | *P*-value† |
|---|---|---|---|---|
| Total | 48 | 288 (17–928) | | |
| Age | | | | |
| Median (quartiles) = 59·8 (55·2–69·8) | 48 | | $r$ = 0·11 | 0·446 |
| GPRC5D gender | | | | |
| Female | 20 | 354 (12–1135) | | 0·950 |
| Male | 28 | 195 (26–873) | | |
| GPRC5D stage ISS | | | | |
| I | 22 | 195 (6–528) | | 0·081 |
| II | 11 | 101 (12–3315) | | |
| III | 15 | 727 (38–1669) | | |
| GPRC5D stage S/D | 47 | | | |
| IA | 6 | 278 (83–357) | | 0·388 |
| II, IIA or IIB | 10 | 35 (3–556) | | |
| IIIA or IIIB | 31 | 437 (15–1301) | | |
| Bone marrow plasma cell infiltration | | | | |
| Median (quartile) = 28·5 (15·0–70·0) | 48 | | $r$ = 0·64 | **<0·001** |
| β2-microglobulin level, mg/L | | | | |
| Median (quartile) = 3·55 (2·70–7·55) | 48 | | $r$ = 0·42 | **0·003** |
| Albumin level, g/L | | | | |
| Median (quartile) = 4·07 (3·61–4·37) | 47 | | $r$ = −0·14 | 0·355 |
| GPRC5D cytogenetics | | | | |
| IgH | | | | |
| Abnormal | 20 | 354 (56–2492) | | 0·174 |
| Normal | 25 | 101 (8–855) | | |
| t(4;14) | | | | |
| Abnormal | 6 | 1922 (375–6081) | | **0·029** |
| Normal | 39 | 101 (12–855) | | |
| t(11;14) | | | | |
| Abnormal | 6 | 57 (18–437) | | 0·392 |
| Normal | 40 | 288 (15–967) | | |
| t(14;16) | | | | |
| Abnormal | 0 | – | | – |
| Normal | 44 | 195 (15–873) | | |

 

**Table 1** *Continued*

| | No. of patients | GPRC5D median (quartiles) | Correlation with GPRC5D* | *P*-value† |
|---|---|---|---|---|
| del rb-1 | | | | |
| Abnormal | 23 | 727 (223–2320) | | **0·003** |
| Normal | 24 | 56 (7–439) | | |
| p53 | | | | |
| Abnormal | 8 | 447 (11–2085) | | 0·926 |
| Normal | 22 | 290 (34–965) | | |

*Spearman's correlation coefficient of each continuous variable with GPRC5D expression.
†*P*-values for continuous variables correspond to Spearman's correlation coefficient, for categorical variables with 2 or 3 categories to Wilcoxon's rank-sum and Kruskal–Wallis test, respectively, with GPRC5D as outcome variable. Statistically significant correlations are shown in bold.

High plasma cell infiltration correlated with high levels of GPRC5D (Spearman's $r = 0{\cdot}64$, $P < 0{\cdot}001$) (Fig. S1). GPRC5D correlated with β2-microglobulin levels (Spearman's $r = 0{\cdot}42$, $P = 0{\cdot}003$) (Fig. S2).

Differences were observed between patients in various subgroups of the international staging system for MM (ISS). Median GPRC5D mRNA expression in stage I was 195 (interquartile range, 6–528), in stage II 101 (12–3315) and in stage III 727 (38–1669). High GPRC5D mRNA expression was associated with higher ISS stage, although not significantly ($P = 0{\cdot}081$) (Table 1). No significant association of GPRC5D with Salmon and Durie stages was observed.

Interphase FISH analysis ($n = 47$) showed chromosomal abnormalities in 34 samples (72·3%), whereas in 13 samples (27·7%), no aberrations were detected. As expected, deletion 13q14 (rb-1) was present in 23 of 47 samples (49%) and IgH translocations in 20 of 45 samples (44%). A translocation t(11;14) (q13;q32) was detected in six of 46 samples (12·5%) and a t(4;14) (p16;q32) in six of 45 samples (13·3%). No t(14;16) was found. In eight of 30 samples, p53 abnormalities were detected.

A significant dependence of GPRC5D mRNA expression was found on deletion 13q14 (rb-1) (median, 727 vs. 56, $P = 0{\cdot}003$) as well as on the t(4;14)(p16;q32) (median, 1922 vs. 101, $P = 0{\cdot}029$). The influence of p53 abnormalities, in eight of 30 samples, on mRNA expression of GPRC5D was statistically not significant (median, 447 vs. 290, $P = 0{\cdot}926$).

## GPRC5D and clinical outcome in multiple myeloma

A significant difference in overall survival was observed in patients with high or low GPRC5D mRNA expression. Figure 2a shows Kaplan–Meier estimates of the distribution of overall survival (OS), where the 48 patients with MM were divided into two groups according to the median of GPRC5D expression (median, 288; $P = 0{\cdot}006$). Median follow-up was 59 months (quartiles, 33–87 months).

A univariate Cox model showed a significant influence of GPRC5D expression on overall survival: every doubling of GPRC5D results in a 14% higher hazard of death (CI, 1–29%; $P = 0{\cdot}031$). However, after adjusting this effect for the potential influence of deletion 13q14 (rb-1) and β2-microglobulin in a multi-variable Cox model, this effect decreases to 11% and is not significant any more. To present this effect of GPRC5D on overall survival in a different way, a patient with GPRC5D expression of 640 is expected to have an about two-thirds higher risk compared with a patient with an expression of 20 ($640/20 = 32 = 2^5$, thus calculating $1{\cdot}11^5 = 1{\cdot}69$).

Furthermore, the univariate Cox model shows a highly significant influence on overall survival by ISS (HR = 5·67 for stage III vs. I) and by β2-microglobulin (HR = 1·85 for each doubling of the β2-MG value), the latter persisting also in the multi-variable model.

Figure 2b shows Kaplan–Meier estimates of the distribution of OS within the subgroup of patients with deletion 13q14 (rb-1), demonstrating a significant influence of GPRC5D expression on overall survival in rb-1-deleted patients. This indicates that GPRC5D is a novel prognostic marker, although with some dependency on other prognostic systems.

## Expression of GPRC5D mRNA in tissue samples and cell lines

To investigate tissue and tumour specificity of GPRC5D, we tested a panel of tissue mRNAs (Fig. 3). Tissue samples showed high expression in testis (142-fold). Lower levels were found in trachea (19-fold), salivary gland (23-fold) and in tonsil (14-fold). In bone marrow and lung tissue, very low levels were detected (3·6-fold and 3·5-fold). Spleen, thyroidal gland and prostate expression ranged from 0·9-fold to 1·5-fold. No expression was found in uterus, spinal cord, kidney, adrenal gland, brain cerebellum, brain (whole), heart, foetal liver, foetal brain, thymus and placenta. No expression was detected in K562, RAMOS, DAUDI, CCRF, Jurkat, REH and RL7.

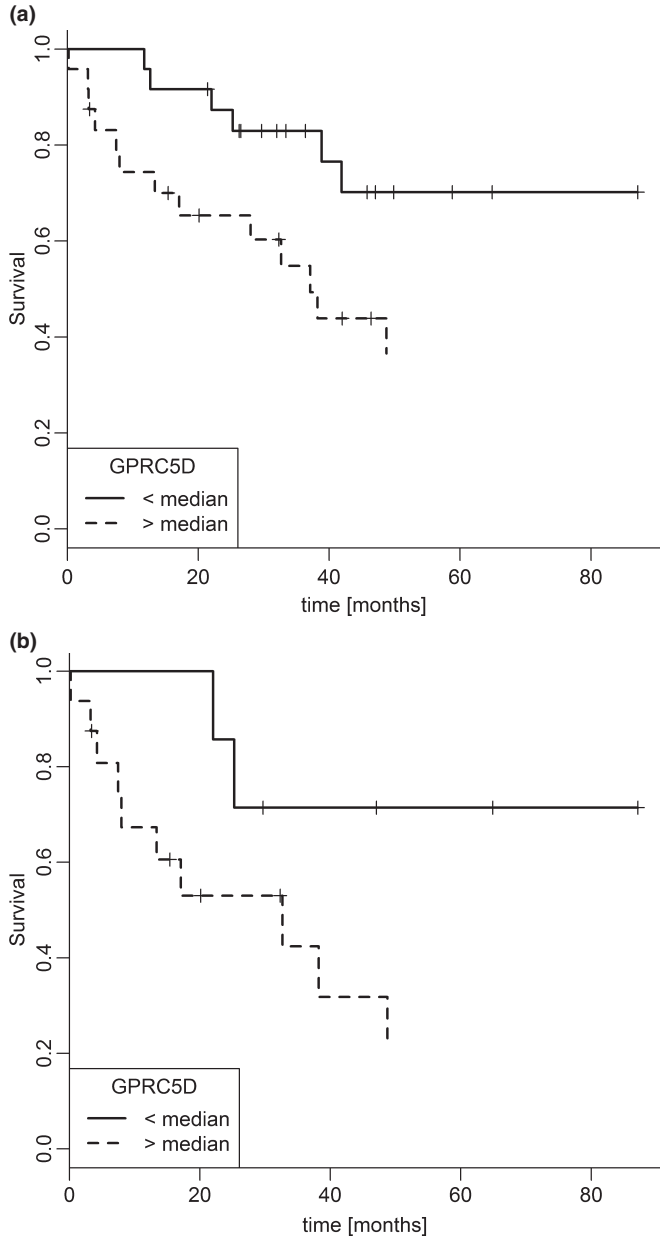


**Figure 2** (a) Kaplan–Meier estimates of the distribution of overall survival. The 48 patients with multiple myeloma were divided into two groups according to the median of GPRC5D expression (median, 288; $P = 0{\cdot}006$). Median follow-up was 59 months (quartiles, 33–87 months). (b) Kaplan–Meier estimates of the distribution of overall survival within the subgroup of patients with deletion 13q14 (rb-1). The blot demonstrates a significant influence of GPRC5D expression on overall survival in rb-1 deleted patients.

## Discussion

This is the first report of the association of the GPRC5D gene with cancer. We show that GPRC5D mRNA is highly expressed in patients with poor-risk MM. High expression was associated with high ISS stage, high β2-microglobulin, deletion 13q14 (rb-1), t(4;14) and interestingly, with high plasma cell count. In multi-variable analysis, the major determinant of outcome was still the serum level of β2-microglobulin. The most striking association of GPRC5D was observed with β2-microglobulin, a single major risk factor in MM [19]. GPRC5D expression showed a significant association with clinical outcome ($P = 0{\cdot}031$). The estimated overall survival of patients expressing GPRC5D above or below the median of 288 was 43·9% vs. 70·2% at 48 months.

Recently, more and more data suggest a crucial role for chromosome 13 as a prerequisite for clonal expansion for tumours [20]. Our study demonstrates that within the subgroup of patients with deletion 13q14 (rb-1) (see Fig. 2b), the effect of GPRC5D on overall survival persists. The estimated overall survival of patients expressing GPRC5D above or below the median of 288 was 43·9% vs. 70·2% at 48 months. Thus, GPRC5D expression might discriminate between poor-and good-risk patients within the rb-1 deleted subgroup. In the light of the ongoing discussion regarding deletion 13q14 (rb-1) and disease progression in MM, our data indicate that the determination of GPRC5D provides valuable additional information [20].

Moreover, there was a highly significant association between mRNA expression of GPRC5D and plasma cell count in bone marrow. While we do not have protein data generated by immunohistochemistry or data on the expression in CD138 selected plasma cells, this suggests that GPRC5D is predominately expressed in plasma cells. This is corroborated by the fact that GPRC5D levels in normal bone marrow were low.

Our data demonstrate overexpression of GPRC5D in the bone marrow of patients with myeloma compared to normal tissues. While the median expression level in poor-risk myeloma, such as ISS stage III, was 727 (range, 38–1669), the median expression in normal tissues was 1 (range, 0–148). Intermediate levels of expression in normal tissues were found in testis, salivary gland, trachea and tonsils (median, 22; range, 14–148). No or very low expression was detected in essential tissues, such as brain, spinal cord, heart, lung, liver, kidney (see Fig. 3). Our findings complement the data published by Bräuner-Osborne *et al.* [13] who found high expression in pancreas (not tested in our panel) and medium expression in kidney, small intestine, spleen and testis. Differences in the relative expression levels might result from differences in the methods used. Moreover, we could not detect relevant expression in B or T cell lines or the myeloid cell line K-562.

 

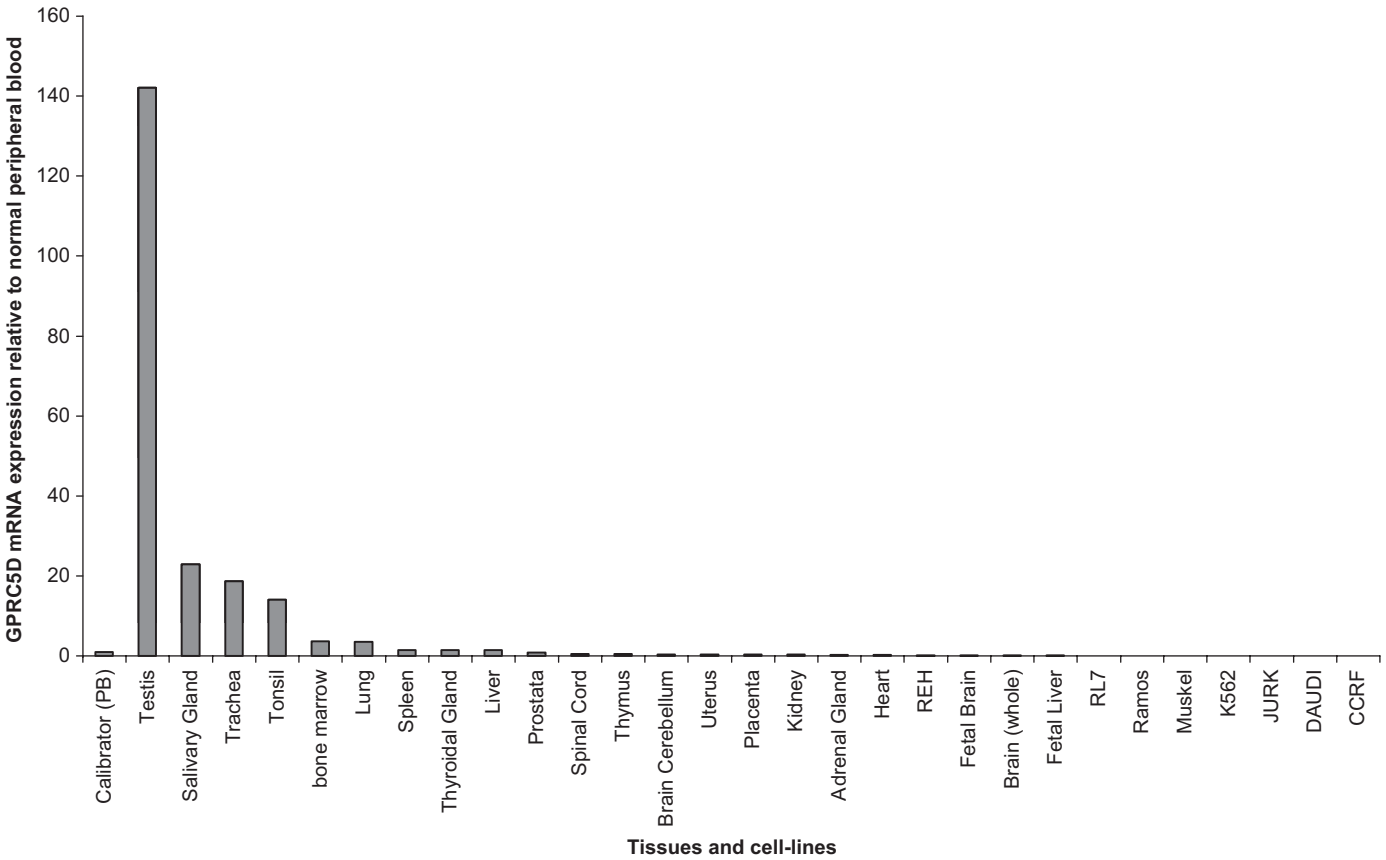


**Figure 3** Expression of GPRC5D in different tissues and cell lines relative to normal peripheral blood. *x*-axis shows all analysed tissues, cell lines and controls. The *y*-axis represents GPRC5D mRNA expression relative to peripheral blood. Tissue samples showed highest results in testis (142-fold). Lower levels were found in salivary gland (23-fold), trachea (19-fold) and in tonsil (14-fold). In bone marrow and lung tissue, very low levels were detected (3·6-fold and 3·5-fold). Spleen, thyroid gland and prostate showed expressions, ranging from 0·9-fold to 1·5 fold. No expressions were found in uterus, spinal cord, kidney, adrenal gland, brain cerebellum, brain (whole), heart, foetal liver, foetal brain, thymus and placenta. In all cell lines, no expressions were detected.

We also explored, whether the GPRC5D protein contains potential T-helper and CTL epitopes based on computerized peptide motifs searches [21,22]. Potential immunogenic peptide sequences were identified according to the known consensus motifs for peptide bound by HLA-A2 and A24 from the naturally occurring GPRC5D sequence [21]. Using the Tsites program, which searches for peptide motifs, several peptide sequences with the potential for eliciting antibody and proliferative T cell responses were identified [22] (data not shown).

Several features identify GPCR5D as a potential novel cancer antigen: (i) overexpression in poor-risk myeloma, (ii) limited low expression in normal tissues, (iii) association with clinical outcome, (iv) cell membrane expression with signalling capabilities [23] and (v) the potential presence of T-helper and CTL epitopes. Other receptors and signalling molecules in lymphoproliferative diseases including MM have successfully been targeted [23,24].

In summary, we have established GPRC5D as a novel prognostic marker in MM. Owing to its unique expression pattern, this molecule could also serve as a potential therapeutic target. Further studies investigating protein expression as well as the function of GPRC5D in MM cells are warranted.

**Acknowledgements**
We thank Uli Zeman for her excellent technical assistance.

**Conflict of interest**
The authors have nothing to disclose and no conflict of interests.

**Address**
Department of Medicine I, Division of Hematology and Hemostaseology, Medical University Vienna, Vienna, Austria (J. Atamaniuk, E. Porpaczy, B. Kainz, B. Hilgarth, H. Gisslinger, U. Jaeger, A. Gaiger); Department of Medical Statistics and Informatics Medical University Vienna, Vienna, Austria (A. Gleiss); Department of Medicine I, Division of Oncology, Medical University Vienna, Vienna, Austria (T. W. Grunt, M. Raderer, J. Drach); Department of Internal Medicine I, Division of Hematology and Oncology, Wilhelminen-Hospital, Vienna, Austria (H. Ludwig).
**Correspondence to:** Prof. Alexander Gaiger, Department of Internal Medicine I, Division of Hematology and Hemostaseology, Medical University Vienna, Austria; Währinger Gürtel 18-20, A-1090 Vienna, Austria. Tel.: +43(0)1 40400 3366; fax: +43(0)1 40400 4030; e-mail: alexander.gaiger@meduniwien.ac.at

Received 11 June 2011; accepted 27 March 2012

## References

1 Blade J, Rosinol L. Changing paradigms in the treatment of multiple myeloma. *Haematologica* 2009;**94**:163–6.
2 Harousseau JL. Induction therapy in multiple myeloma. *Hematology Am Soc Hematol Educ Program* 2008;**1**:306–12.
3 Blade J, Rosinol L. Advances in therapy of multiple myeloma. *Curr Opin Oncol* 2008;**20**:697–704.
4 Raab MS, Podar K, Breitkreutz I, Richardson PG, Anderson KC. Multiple myeloma. *Lancet* 2009;**374**:324–39.
5 Kettle JK, Finkbiner KL, Klenke SE, Baker RD, Henry DW, Williams CB. Initial therapy in multiple myeloma: investigating the new treatment paradigm. *J Oncol Pharm Pract* 2009;**10**:10.
6 Berenson JR, Yellin O. New drugs in multiple myeloma. *Curr Opin Support Palliat Care* 2008;**2**:204–10.
7 Podar K, Tai YT, Hideshima T, Vallet S, Richardson PG, Anderson KC. Emerging therapies for multiple myeloma. *Expert Opin Emerg Drugs* 2009;**14**:99–127.
8 Maiso P, Ocio EM, Garayoa M, Montero JC, Hofmann F, Garcia-Echeverria C *et al*. The insulin-like growth factor-I receptor inhibitor NVP-AEW541 provokes cell cycle arrest and apoptosis in multiple myeloma cells. *Br J Haematol* 2008;**141**:470–82.
9 Hewish M, Chau I, Cunningham D. Insulin-like growth factor 1 receptor targeted therapeutics: novel compounds and novel treatment strategies for cancer medicine. *Recent Pat Anticancer Drug Discov* 2009;**4**:54–72.
10 Ocio EM, Maiso P, Chen X, Garayoa M, Alvarez-Fernandez S, San-Segundo L *et al*. Zalypsis: a novel marine-derived compound with potent antimyeloma activity that reveals high sensitivity of malignant plasma cells to DNA double-strand breaks. *Blood* 2009;**113**:3781–91.
11 Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. *Leukemia* 2009;**23**:3–9.
12 Heintel D, Zojer N, Schreder M, Strasser-Weippl K, Kainz B, Vesely M *et al*. Expression of MUM1/IRF4 mRNA as a prognostic marker in patients with multiple myeloma. *Leukemia* 2008;**22**:441–5.
13 Brauner-Osborne H, Jensen AA, Sheppard PO, Brodin B, Krogsgaard-Larsen P, O'Hara P. Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D. *Biochim Biophys Acta* 2001;**1518**:237–48.
14 Inoue S, Nambu T, Shimomura T. The RAIG family member, GPRC5D, is associated with hard-keratinized structures. *J Invest Dermatol* 2004;**122**:565–73.
15 Xu J, Tian J, Shapiro SD. Normal lung development in RAIG1-deficient mice despite unique lung epithelium-specific expression. *Am J Respir Cell Mol Biol* 2005;**32**:381–7.
16 Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, Blade J *et al*. International staging system for multiple myeloma. *J Clin Oncol* 2005;**23**:3412–20.
17 Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996;**17**:343–6.
18 Simera I, Moher D, Hoey J, Schulz KF, Altman DG. A catalogue of reporting guidelines for health research. *Eur J Clin Invest* 2010;**40**:35–53.
19 Kapoor P, Kumar S, Fonseca R, Lacy MQ, Witzig TE, Hayman SR *et al*. Impact of risk stratification on outcome among patients with multiple myeloma receiving initial therapy with lenalidomide and dexamethasone. *Blood* 2009;**114**:518–21.
20 Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK *et al*. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. *Leukemia* 2009;**23**:2210–21.
21 Feller D, Cruz VF. Identifying antigenic T cell sites. *Nature* 1991;**349**:720–1.
22 Rothbard JB, Taylor WR. A sequence pattern common to T cell epitopes. *EMBO J* 1988;**7**:93–100.
23 Laubach JP, Mahindra A, Mitsiades CS, Schlossman RL, Munshi NC, Ghobrial IM *et al*. The use of novel agents in the treatment of relapsed and refractory multiple myeloma. *Leukemia* 2009;**10**:10.
24 Kyle RA, Rajkumar SV. Treatment of multiple myeloma: a comprehensive review. *Clin Lymphoma Myeloma* 2009;**9**:278–88.

## Supporting Information

Additional supporting information can be found in the online version of this article:

**Figure S1.** Spearman's correlation coefficient between GPRC5D expression and plasma cell %: $r = 0.64$; $P < 0.001$.

**Figure S2.** Spearman's correlation coefficient between GPRC5D expression and β2-microglobulin (mg/L): $r = 0.42$; $P < 0.003$.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

 