Transcriptional analysis of BMP2 and BMP6 in patients with newly diagnosed multiple myeloma

PANAGIOTIS STOIKOS[1], ANGELOS GIANNAKOULAS[1,2], EVANGELIA KOUVATA[2], GEORGIA STEFANI[2], GEORGE VASSILOPOULOS[2] and NIKOLAOS GIANNAKOULAS[2]

[1]Laboratory of Hematology Department, Faculty of Medicine, University of Thessaly, 41110 Larissa, Greece; [2]Hematology Department, University Hospital of Larissa, 41110 Larissa, Greece

Received February 20, 2025; Accepted July 1, 2025

DOI: 10.3892/ol.2025.15194

Abstract. Multiple myeloma (MM) is a highly heterogenous disease with diverse outcomes across patients. Stratifying patients is a promising approach to predict their clinical outcomes. However, despite notable progress in stratification systems, there is still a need for further optimization. Combining the existing staging parameters with molecular approaches could offer a better understanding of 'high risk' disease and guide treatment decisions. Bone morphogenetic proteins (BMPs) represent a group of proteins, which are members of the TGF-β superfamily that are now considered to be multifunctional cytokines. BMPs are mostly differentiation factors that are involved in bone metabolism, tissue development and carcinogenesis. In MM, BMP signaling is less well characterized and its association with the pathogenesis of MM is still under investigation. Therefore, the present study aimed to evaluate BMP2 and BMP6 expression and to study the clinical impact of their expression. BMP2 and BMP6 expression was quantified using reverse transcription-quantitative PCR post-RNA extraction from bone marrow mononuclear cells derived from patients with MM and healthy donors. According to the observations in a group of patients with newly diagnosed MM, neither BMP2 nor BMP6 were able to serve as prognostic biomarkers for patients.

Introduction

Multiple myeloma (MM) is an incurable malignant plasma cell disorder characterized by the infiltration of clonal plasma cells in the bone marrow compartment. It is the second most common hematologic malignancy with an estimated incidence of 5/100.000 (1). The introduction of novel agents has significantly prolonged the survival of patients with MM and enhanced their quality of life (2).

Despite significant progress in the therapeutic landscape of MM, there remains a proportion of patients, considered as 'high risk' patients, who do not exhibit favorable outcomes (3). Identifying patients with 'high risk' MM is crucial for clinicians since these patients may benefit from an alternative, more intense therapeutic approach. In everyday practice, patient stratification is based on the ISS and R^2-ISS staging systems, on cytogenetic abnormalities and common laboratory findings. Combining these existing approaches with molecular approaches may offer an improved understanding of high-risk disease and optimize the recognition of such patients.

Bone morphogenetic proteins (BMPs) represent a group of pleiotropic proteins, members of the TGF-β superfamily, that are now considered multifunctional cytokines. Extensive research in the previous decades has revealed that BMPs are typically differentiation factors that are involved in bone metabolism, tissue development and carcinogenesis (4).

To date, ≥20 members of the BMP family have been identified (5). Their activity is mediated upon binding as homodimers or heterodimers to their respective serine/threonine kinase receptors and ultimately their signal is intracellularly transduced via SMAD proteins. The diverse effects that are observed among different BMPs imply a strict regulation of their activity. It is hypothesized that this strict regulation is achieved through ligand's availability, through the concurrent activation or inactivation of different BMP molecules, through up- or down-regulation of BMP receptors and through the presence of inhibitory or stimulatory molecules (5).

In MM, BMP signaling is not as well characterized and its association with the pathogenesis of MM is still poorly understood. The non-tumor-specific mechanism of action of BMPs is probably the reason for the limited interest and data of this pathway in MM, resulting in the lack of knowledge regarding their prognostic significance. Previous studies attempting to assess endogenous expression of BMPs in myeloma cells reported elevated expression of BMP4 and BMP6 in patients with MM compared to healthy donors (HD) (6,7). Similarly, serum analysis found increased protein expression levels of BMP9 and BMP2 in patients with MM compared to controls (8,9).

Correspondence to: Dr Nikolaos Giannakoulas, Hematology Department, University Hospital of Larissa, Viopolis, 41110 Larissa, Greece
E-mail: ngiannak@med.uth.gr; ngiannakoulas@hotmail.com

Key words: multiple myeloma, prognostic biomarkers, bone morphogenetic protein 2, bone morphogenetic protein 6

Prompted by the known tumor-inductive effects of BMP2 and BMP6 in several cancer types (10-13), the expression of BMP2 and BMP6 in a cohort of MM patients was assessed and the clinical impact of their expression was determined.

Patients and methods

Study design and patients. This was a retrospective study aiming to assess the clinical significance of BMP2 and BMP6 in patients with newly diagnosed multiple myeloma. Study endpoints were to evaluate the expression levels of BMP2 and BMP6 in NDMM and to determine whether BMP2 or BMP6 could serve as prognostic biomarkers for patients with NDMM. The study enrolled NDMM patients that were diagnosed and treated in the Department of Hematology of University Hospital of Larissa, Greece. Diagnosis of MM was based on the International Myeloma Working Group consensus criteria (14). The enrollment period was from March 2019 to December 2021. Nine healthy volunteers (male 5, median age 60, range 51-75) were additionally enrolled, at the same period of time. All participants provided written informed consent, prior to recruitment, to the use of their biological samples and clinical data in research and the study was reviewed and approved by the Institutional Review Board of the University Hospital of Larissa (approval code 21906) and adhered to the tenets of The Declaration of Helsinki. Clinical and laboratory data (collected prospectively) were retrieved from the electronic database and biological samples were retrieved from the department's biobank. All samples were acquired at the time of diagnosis and prior to treatment initiation.

Sampling, RNA extraction, cDNA synthesis and quantitative PCR (qPCR). Bone marrow aspirates were collected in EDTA tubes and immediately processed (within 2 h post sampling). Bone marrow mononuclear cells (BMMNCs) were isolated using density gradient separation with a Ficoll Paque Plus kit (MilliporeSigma) (Fig. S1). Bone marrow was diluted 1:1 with DPBS (Biowest) and was filtered through a 100 μm pore to remove cell clumps, clots and bone fragments. 4 ml of diluted cell suspension was carefully layered over 4 ml of Ficoll Paque Plus and an immediate centrifugation step (400 x g at 20˚C for 30 min without brake) followed. The upper plasma layer was aspirated and the interphase was drawn for washing steps and further analyses. Cells were then washed with DPBS (Biowest), lysed with red blood cell lysis buffer (Cell Signaling Technology, Inc.) if required, aliquoted and stored as dry pellets at -80˚C until further analysis. Total RNA was retrieved from BMMNCs using an E.Z.N.A. Total RNA kit I (Omega-Biotek Inc.) according to manufacturer's instructions. Briefly, $5x10^6$ pelleted cells were lysed using TRK lysis buffer and homogenized with a syringe. The lysate was then filtered through columns to remove debris and other contaminants. Washing steps were applied along with a middle DNA-digestion step and high-yield RNA was eventually eluted in 30 μl nuclease-free water. RNA concentration was quantified at 260 nm on a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). Prior to cDNA synthesis, purity and integrity of eluted RNA samples was verified by 1% agarose gel electrophoresis and by A260/280 (median ratio 2.08, range 1.8-2.14)-A260/230 (median ratio 1.93, range 1.4-2.2) absorbance ratios. As expected, agarose gel electrophoresis of RNA displayed the presence of 2 ribosomal bands (28S and 18S) with no smearing or any DNA band and the absorbance ratios were within accepted bounds (15-19). Total RNA was subsequently reverse transcribed into cDNA in a 20 μl reaction volume using a QuantiTect Reverse Transcription kit (Qiagen, GmbH). Approximately, 100 μg RNA template was incubated at 42˚C with dNTPs, RNAse inhibitor, Quantiscript's Reverse Transcriptase, RT Buffer, RT Primer Mix along with RNase-free water. An initial incubation of 3 min at 95˚C was applied to activate Quantiscript Reverse Transcriptase. cDNA was diluted and then deposited at -20˚C. qPCR was carried out in a 36 well Rotor Gene Q (Qiagen) with CYBR green 1 as fluorescent dye. B-2 microglobulin (B2M) was used as housekeeping gene. Intra-spanning primers with amplicon lengths of around 100 bp (acquired from Qiagen GmbH, GeneGlobe ID: SBH0312240 for B2M, GeneGlobe ID: SBH0638609 for BMP2 and GeneGlobe ID: SBH0513495 for BMP6) were used to ensure that traces of genomic DNA would not affect the PCR. Template cDNA was mixed with QuantiNova Probe PCR Master Mix and RNase-free water with volumes according to the manufacturer's protocol. The cycling conditions included an initial incubation step at 95˚C for 2 min followed by 40 cycles of 95˚C for 5 sec and 60˚C for 10 sec. Specificity of each reaction was confirmed by resolving amplification products in a 2% agarose gel, stained with ethidium bromide (Fig. S2). Appropriate sample handling was verified by performing PCR reactions in duplicates. Cq values are presented as means. When for a specific sample the disagreement between the Cq measurements was greater than 1, the qPCR was performed for a third time and the final Cq value was calculated as the mean of the 3 measurements. Gene expression levels were analyzed using the Livak $2^{-\Delta\Delta Cq}$ method (20), after validating stable expression of B2M gene between patients and healthy individuals (median raw Cq value of patients 16.3, range 13.2-24.2 and median raw Cq value of healthy donors 18.5, range 14.4-19.8, P-value 0.1).

Statistical analysis. Statistical analysis was performed with the GraphPad Prism version 10 (Dotmatics). Results were considered significant when P-values were <0.05. The normality of distribution was assessed using a Shapiro-Wilk test. For non-parametric data, a Mann Whitney U test was used to compare differences between two groups. For parametric data, an unpaired Student's t-test was applied. The Fisher's exact test was used to identify differences between the proportions of categories in two independent groups. Univariate and multivariate analyses were carried out by Cox regression analysis. Pearson correlation was performed to assess the linear relationship between two continuous variables and Spearman correlation was used for categorical data. Kaplan-Meier (KM) curves were applied to plot survival and progression-free survival. The log-rank test was used to compare the distribution of time to event between 2 groups.

Results

Patient characteristics. A total of 18 newly diagnosed patients with MM were included in the present study along with 9 additional healthy volunteers who served as controls (male

Table I. Patient characteristics.

Characteristic	Value
Median age, years (range)	62 (44-86)
Sex, n (%)	
Male	9 (50%)
Female	9 (50%)
MM type, n (%)	
IgG	9 (50.0%)
IgA	6 (33.3%)
IgM	1 (5.6%)
Light chain only	2 (11.1%)
Median bone marrow infiltration, % (range)	55 (10-90)
ISS, n (%)	
1	3 (16.7%)
2	7 (38.9%)
3	8 (44.4%)
R^2-ISS, n (%)	
1	3 (16.7%)
2	3 (16.7%)
3	10 (55.5%)
4	2 (11.1%)
Cytogenetic risk, n (%)	
Standard	13 (72.2%)
High	5 (27.8%)
Lytic bone lesions, n (%)	
None	5 (27.8%)
1-3	5 (27.8%)
>3	8 (44.4%)
Skeletal-related events at diagnosis, n (%)	
Fractures	7 (38.9%)
Bone related RT or surgery	6 (33.3%)
Plasmacytoma's at diagnosis, n (%)	6 (33.3%)
Frontline treatment, n (%)	
VCD	7 (38.9%)
VRD	7 (38.9%)
RD	2 (11.1%)
VRD-Autologous	2 (11.1%)

MM, multiple myeloma; ISS, International Staging System; R^2-ISS, revised squared ISS; VCD, Bortezomib-Cyclophosphamide-Dexamethasone; VRD, Bortezomib-Lenalidomide-Cyclophosphamide; RD, Lenalidomide-Dexamethasone.

5, median age 60 years, range 51-75 years). Patient baseline characteristics are shown in Table I. Of the patients with MM, the median age was 62 years, 50% were male and most had IgG MM (50%). The median percentage of bone marrow infiltration was 55% (range 10-90%) and the median beta-2 microglobulin levels were 4.9 mg/l (range 2.3-14.1). A total of 33% of patients presented with elevated LDH levels. High risk cytogenetics were observed in 5 patients (27%). Regarding the ISS staging, 16% were ISS-1, 38% were ISS-2 and 44% were ISS-3. The majority of patients presented with bone disease (72%). The median follow-up period was 50 months (range 4-67). Patients were primarily treated either with VCD or VRD as induction therapy and response was evaluated after five cycles of upfront therapy.

Expression of BMP2 and BMP6. To assess whether BMP2 and BMP6 are differentially expressed in multiple myeloma, the expression levels of BMP2 and BMP6 genes between NDMM patients and healthy controls were assessed. BMP6 mRNA abundance was 5-fold higher in the NDMM group compared to the HD group (unpaired Student's t-test, P=0.0004) (Fig. 1A). In contrast, BMP2 expression was not significantly different between the two groups (Mann Whitney U test, P=0.5) (Fig. 1B). Based on these results, BMP6 was further studied regarding the clinical impact of its expression. Patients were categorized into two groups based on the expression levels of BMP6. The median ΔCq value of the NDMM group was used as the threshold to divide the patients into high and low BMP6 expression groups, each with 9 patients. In the high expression group, the median ΔCq value was 10.1 (range 9.2-11) while in the low expression group the median ΔCq value was 11.8 (range 11.2-13.7). The characteristics of each group are summarized in Table II.

Predictive value of BMP6. The overall response rate (≥PR) was 77% (n=7/9) for the high expression group and 88% (n=8/9) for the low expression group, whereas the ≥ VGPR rate was 55% (n=5/9) and 44% (n=4/9), respectively. Response patterns are shown in Fig. 1C. The median progression free survival (calculated using the Kaplan Meier curves) was 30 months for the high expression group compared to 46 months for the low expression group (log-rank HR=1.6, 95% CI: 0.4-5.3, P=0.3) (Fig. 1D). The median estimated overall survival was 42 months for the high expression group and was not reached for the low expression group (log-rank HR=1.9, 95% CI: 0.4-7.8, P=0.4) (Fig. 1E). In the univariate analysis, age (P=0.06, HR=1.06, 95% CI: 0.99-1.13), LDH levels (P=0.2, HR=1.006, 95% CI: 0.99-1.01), percentage of bone marrow infiltration (P=0.3, HR=3.9, 95% CI: 0.1-80), b2-microglobulin levels (P=0.5, HR=1.07, 95% CI: 0.8-1.3) and ΔCq values of BMP6 (P=0.1, HR=0.6, 95% CI: 0.3-1.1) were factors affecting survival. Whereas, for most factors statistical significance was not reached, they are described as affecting survival based on the hazard ratio values. In the multivariate analysis, age (P=0.05, HR=1.07, 95% CI: 0.99-1.16) was the only factor affecting survival. Pearson and Spearman correlation analyses (Fig. S3) did not show any correlation between BMP6 ΔCq values and age (r=0.02, P=0.9), LDH levels (r=-0.08, P=0.7), b2-microglobulin levels (r-0.2, P=0.4), percentage of bone marrow infiltration (r=-0.1, P=0.4) and ISS stage (r_s=0.1, P=0.6) thus limiting the confounding factors for the subgroup analysis.

BMP6 and bone disease. Next, whether BMP6 expression affected patient's bone status at diagnosis was assessed, given that the BMP family of proteins is a crucial signaling cascade for bone homeostasis and that BMP6 specifically is an osteogenic mediator (21,22). The presence of more than 3 spinal osteolytic lesions was used as the cut-off point to divide the cohort into patients with highly active bone disease and patients with less active bone disease. A total of 33%

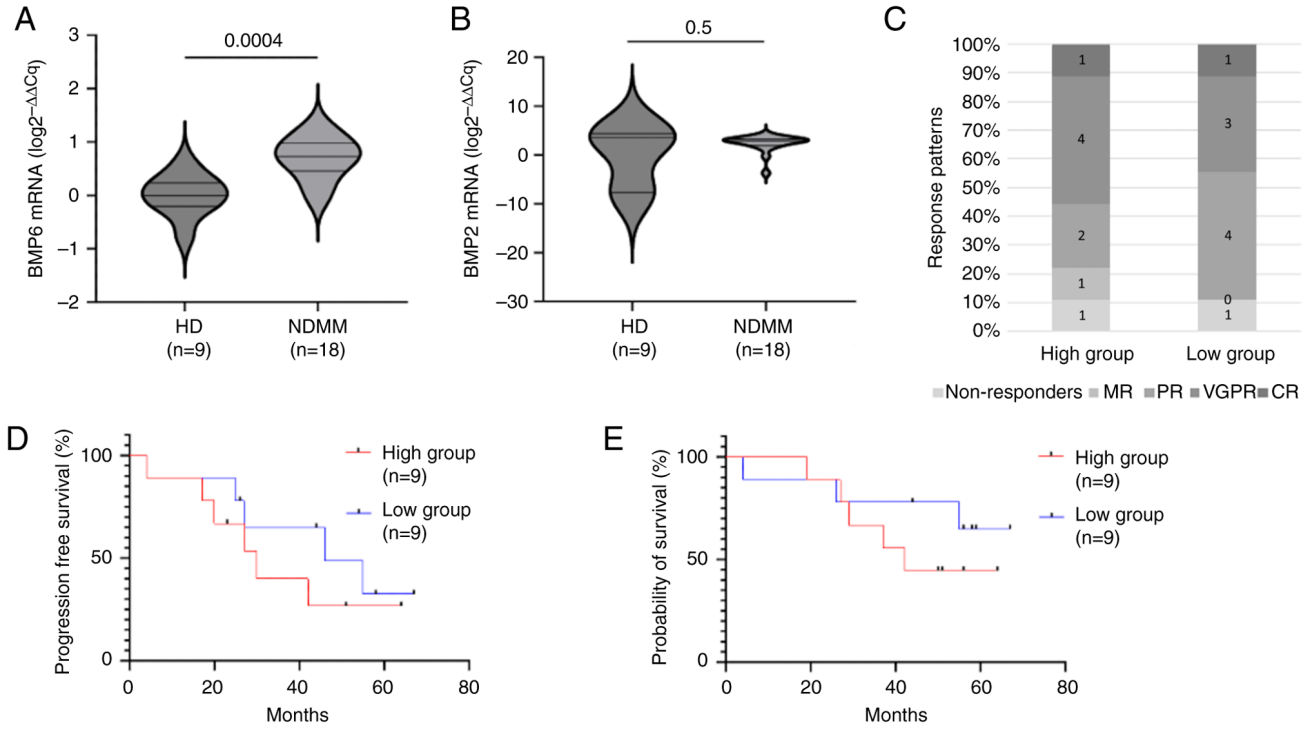

Figure 1. Transcriptional analysis of BMP2 and BMP6 in MM. Relative gene expression analysis of (A) BMP6 and (B) BMP2 between patients with NDMM and healthy controls. (C) Response patterns between patients with elevated BMP6 expression values (High group) and patients with lower BMP6 values (Low group). Response was evaluated post 5 cycles of induction therapy. Comparison of (D) progression free survival and (E) probability of survival in the High and Low expression groups. BMP, bone morphogenetic protein; MM, multiple myeloma; NDMM, newly diagnosed multiple myeloma; HD, healthy donors; CR, complete response; VGPR, very good partial response; PR, partial response; MR, minimal response.

(n=3/9) of patients from the high BMP6 expression group exhibited highly active bone disease compared to 55% (n=5/9) of patients from the low BMP6 expression group (Fisher's exact test, P=0.6). Similarly, the rate of skeletal-related-events (based on the presence of at least one fracture, the need for bone-related radiotherapy and the need for bone-related surgery) was 22% (n=2/9) for the high BMP6 expression group and 55% (n=5/9) for the low BMP6 expression group (Fisher's exact test, P=0.3).

Discussion

MM is traditionally considered a highly heterogenous disease with distinct subtypes (23,24). Each subtype is characterized by unique genetic and molecular abnormalities. This diversity is the primary reason for the diverse outcomes observed in patients. Stratifying patients, based on their risk, is a promising approach to guide treatment decision and treatment intensity. Despite significant progress in stratification systems, there is still a great need for further optimization. Combining existing staging parameters with molecular approaches may offer a better understanding of 'high risk' disease and guide therapeutic protocols.

In the present study, whether bone morphogenetic protein 2 and 6 could serve as biomarkers and whether they could be used to predict clinical outcomes of patients with MM was assessed. The study enrolled 18 newly diagnosed patients and 9 age- and sex-matched healthy donors. The expression levels of BMP2 and BMP6 genes between patients and donors were first assessed. ELISA detection was not performed in the present study due to lack of stored samples. While the expression of BMP6 was significantly different between the 2 groups, this was not the case for BMP2. Thus, only BMP6 was used for subsequent analyses.

The present study used B2M as housekeeping gene. In general, B2M is an acceptable housekeeping gene which is considered to have a stable and ubiquitous expression across species and cells (25) but usually other genes such as ACTB and GAPDH are more preferable for normalization. To verify the stable expression of B2M as housekeeping gene, the raw Cq values of B2M between patients (median Cq value 16.3, range 13.2-24.2) and healthy donors (median Cq value 18.5, range 14.4-19.8) were compared and no significant difference between the 2 groups was found (P-value=0.1). The interquartile range of B2M's raw Cq values was 2.4 for healthy donors and 6.2 for patients indicating that the Cq deviation was mostly affected from the outliers. Verification of results with other housekeeping genes was not performed due to lack of residual samples for some of the patients. Literature review indicated that the observed expression pattern of B2M of this study is in alliance with previous studies (25-28). Thus, subsequent analysis was performed.

The patient cohort was then divided into two subgroups based on the expression values of BMP6. The median ΔCq served as the cut-off point. The first subgroup included patients with elevated expression measures of BMP6 and the second subgroup consisted of patients with lower expression measures. Both groups consisted of patients with similar characteristics. Comparative analysis between the two subgroups did not show

Table II. Characteristics of each patient group, stratified according to BMP6 expression.

Characteristic	High BMP6 Group (n=9)	Low BMP6 Group (n=9)
Median age, years (min-max)	59 (44-84)	63 (52-86)
Sex (M/F)	5/4	4/5
ISS stage		
ISS stage 1	2	1
ISS stage 2	3	4
ISS stage 3	4	4
R^2-ISS stage		
R^2-ISS stage 1	2	1
R^2-ISS stage 2	2	1
R^2-ISS stage 3	4	6
R^2-ISS stage 4	1	1
Median bone marrow infiltration, % (min-max)	60 (15-80)	50 (10-90)
Presence of high-risk cytogenetics	3	2
Frontline treatment		
VCD	4	3
VRD	2	5
RD	1	1
VRD-Autologous	2	0

BMP, bone morphogenetic protein; ISS, International Staging System; R^2-ISS, revised squared ISS; VCD, Bortezomib-Cyclophosphamide-Dexamethasone; VRD, Bortezomib-Lenalidomide-Cyclophosphamide; RD, Lenalidomide-Dexamethasone.

any significant difference for response rates, progression free survival and overall survival.

Bone morphogenetic proteins represent a large family of proteins with pleiotropic actions both under basal and abnormal conditions. Previous studies have delineated the significant role of BMPs in cancer. Paradoxically, BMPs can act both as tumor promoters and tumor suppressors (29). It is reasonable to assume that this duality reflects their tumor-specific mechanism of action but others have suggested that their role is ultimately determined by their receptors, by inhibitory or stimulatory molecules and by the concurrent activation and/or inactivation of other BMPs.

Up till now, the role of BMP signaling in MM pathogenesis remains unclear with contradictory findings from previous studies. In the present study, the expression of BMP2 and BMP6 in a cohort of NDMM patients was assessed and the correlation between their expression and clinical outcomes was determined. BMP2, particularly, is of great interest since it is implied to promote lung cancer adenocarcinoma migratory ability via BMP2 receptor activation and subsequent SMAD 1/5/8 phosphorylation, independently of KRAS pathway activation (10). Although, both BMP2 and BMP6 were expressed by patient samples, only the expression of BMP6 was significantly upregulated in patients compared to healthy donors. This is inconsistent with Maes *et al* (9) who reported significantly different BMP2 serum protein levels between patients with MM and controls. It is hypothesized that the non-linear relationship between mRNA and protein expression could be a possible explanation for the discrepancy between the present and past studies.

Consistent with what is known, the expression levels of BMP6 were significantly elevated in MM samples, although its expression failed to stratify patients. The relatively small ΔCq range among patients could be a reason for that failure implying that a different lab approach, could offer superior results. The absolute quantification of BMP6 mRNA levels or the serum measurement of BMP6 protein could be alternative options. Mechanistically, increased BMP6 expression is considered favorable as it inhibits MM growth and development. Seckinger *et al* (7) reported that the treatment of human myeloma cell lines with exogenous BMP6 inhibited proliferation of cultured cells. Similarly, it was recently demonstrated that the inhibitory effect of BMP6 was mediated through the ALK-2 receptor (30). The anti-myeloma effect of BMP6 has also been observed in in-vitro models where it was observed to inhibit tubule formation, thus highlighting a potential role of BMP6 in angiogenesis and MM in general (7). In contrast to MM, BMP6 is considered as a tumor inductive molecule in breast cancer. It was reported that BMP6 inhibited MDA-MB-23 cell line apoptosis via p38 and survivin activation (12).

In conclusion, based on the results of the present study on patients with NDMM, neither BMP2 nor BMP6 accomplished to serve as biomarkers for patients. The results indicate that BMP2 and BMP6 may not be involved in MM's pathogenesis. However, the present study is limited by the small sample size, which may have affected the results obtained and conclusions drawn, indicating that additional large-scale studies are needed for safer assumptions. Additionally, it is possible that other members of the BMP family may influence MM growth and development. Apart from the potential role of BMPs in

patient stratification, future studies should emphasize in the mechanistic exploration of BMP signaling in MM. Knockdown assays could, for starters, explain whether BMPs role is ultimately determined by their receptors or by the concurrent activation and/or inactivation of other BMPs.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

PS performed experiments. PS, GS and EK collected data. AG and EK analyzed data. AG wrote the initial manuscript. GV and NG revised the manuscript. NG designed the study. GV contributed to study conception and design. GV and NG supervised the study. GV and NG confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided written informed consent prior to recruitment, and the study was reviewed and approved by the Institutional Review Board of the University Hospital of Larissa (approval code 21906) and adhered to the tenets of The Declaration of Helsinki.

Patient consent for publication

All participants provided written informed consent for their information to be published.

Competing interests

The authors declare no competing interests.

References

1. Ludwig H, Novis Durie S, Meckl A, Hinke A and Durie B: Multiple Myeloma incidence and mortality around the globe; Interrelations between health access and quality, economic resources, and patient empowerment. Oncologist 25: e1406-e1413, 2020.
2. Eisfeld C, Kajüter H, Möller L, Wellmann I, Shumilov E and Stang A: Time trends in survival and causes of death in multiple myeloma: A population-based study from Germany. BMC Cancer 23: 317, 2023.
3. Rajkumar SV: Multiple myeloma: 2022 update on diagnosis, risk stratification, and management. Am J Hematol 97: 1086-1107, 2022.
4. Katagiri T and Watabe T: Bone morphogenetic proteins. Cold Spring Harb Perspect Biol 8: a021899, 2016.
5. Bragdon B, Moseychuk O, Saldanha S, King D, Julian J and Nohe A: Bone morphogenetic proteins: A critical review. Cell Signal 23: 609-620, 2011.
6. Grcević D, Kusec R, Kovacić N, Lukić A, Lukić IK, Ivcević S, Nemet D, Seiwerth RS, Ostojić SK, Croucher PI and Marusić A: Bone morphogenetic proteins and receptors are over-expressed in bone-marrow cells of multiple myeloma patients and support myeloma cells by inducing ID genes. Leuk Res 34: 742-751, 2010.
7. Seckinger A, Meissner T, Moreaux J, Goldschmidt H, Fuhler GM, Benner A, Hundemer M, Rème T, Shaughnessy JD Jr, Barlogie B, *et al*: Bone morphogenic protein 6: A member of a novel class of prognostic factors expressed by normal and malignant plasma cells inhibiting proliferation and angiogenesis. Oncogene 28: 3866-3879, 2009.
8. Olsen OE, Wader KF, Misund K, Våtsveen TK, Rø TB, Mylin AK, Turesson I, Størdal BF, Moen SH, Standal T, *et al*: Bone morphogenetic protein-9 suppresses growth of myeloma cells by signaling through ALK2 but is inhibited by endoglin. Blood Cancer J 4: 196, 2014.
9. Maes K, Nemeth E, Roodman GD, Huston A, Esteve F, Freytes C, Callander N, Katodritou E, Tussing-Humphreys L, Rivera S, *et al*: In anemia of multiple myeloma, hepcidin is induced by increased bone morphogenetic protein 2. Blood 116: 3635-3644, 2010.
10. Wu CK, Wei MT, Wu HC, Wu CL, Wu CJ, Liaw H and Su WP: BMP2 promotes lung adenocarcinoma metastasis through BMP receptor 2-mediated SMAD1/5 activation. Sci Rep 12: 16310, 2022.
11. Raida M, Clement JH, Leek RD, Ameri K, Bicknell R, Niederwieser D and Harris AL: Bone morphogenetic protein 2 (BMP-2) and induction of tumor angiogenesis. J Cancer Res Clin Oncol 131: 741-750, 2005.
12. Du J, Yang S, Wang Z, Zhai C, Yuan W, Lei R, Zhang J and Zhu T: Bone morphogenetic protein 6 inhibit stress-induced breast cancer cells apoptosis via both Smad and p38 pathways. J Cell Biochem 103: 1584-1597, 2008.
13. Stieglitz D, Lamm S, Braig S, Feuerer L, Kuphal S, Dietrich P, Arndt S, Echtenacher B, Hellerbrand C, Karrer S and Bosserhoff AK: BMP6-induced modulation of the tumor micro-milieu. Oncogene 38: 609-621, 2019.
14. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Kumar S, Hillengass J, Kastritis E, Richardson P, *et al*: International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 15: 538-548, 2014.
15. Fleige S and Pfaffl MW: RNA integrity and the effect on the real-time qRT-PCR performance. Mol Aspects Med 27: 126-139, 2006.
16. Giraldo-Parra L, Ramirez LG, Navas A and Gómez MA: Quality parameters for RNA preparations from biopsies of ulcerated human skin. Wellcome Open Res 28: 249, 2023.
17. Okamoto T and Okabe S: Ultraviolet absorbance at 260 and 280 nm in RNA measurement is dependent on measurement solution. Int J Mol Med 5: 657-659, 2000.
18. Pinto FL, Thapper A, Sontheim W and Lindblad P: Analysis of current and alternative phenol based RNA extraction methodologies for cyanobacteria. BMC Mol Biol 7: 79, 2009.
19. Wilfinger WW, Mackey K and Chomczynski P: Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22: 478-481, 1997.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. Ebisawa T, Tada K, Kitajima I, Tojo K, Sampath TK, Kawabata M, Miyazono K and Imamura T: Characterization of bone morphogenetic protein-6 signaling pathways in osteoblast differentiation. J Cell Sci 112: 3519-27, 1999.
22. Xu H, Tong G, Yan T, Dong L, Yang X, Dou D, Sun Z, Liu T, Zheng X, Yang J, *et al*: Transcriptomic analysis provides insights to reveal the bmp6 function related to the development of intermuscular bones in zebrafish. Front Cell Dev Biol 10: 821471, 2022.
23. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, Epstein J, Yaccoby S, Sawyer J, Burington B, *et al*: The molecular classification of multiple myeloma. Blood 108: 2020-2028, 2006.
24. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, Morgan G, Van Ness B, Chesi M, Minvielle S, *et al*: International myeloma working group. International myeloma working group molecular classification of multiple myeloma: spotlight review. Leukemia 23: 2210-2221, 2009.
25. Matsuzaki Y, Umemoto T, Tanaka Y, Okano T and Yamato M: β2-Microglobulin is an appropriate reference gene for RT-PCR-based gene expression analysis of hematopoietic stem cells. Regen Ther 23: 91-97, 2015.

26. Sabbir H: Validation of internal control genes for quantitative real-time PCR under different experiment conditions in Multiple Myeloma. J Bio Sci Biotechnol 7: 79-90, 2019.
27. Nazari F, Parham A and Maleki AF: GAPDH, β-actin and β2-microglobulin, as three common reference genes, are not reliable for gene expression studies in equine adipose- and marrow-derived mesenchymal stem cells. J Anim Sci Technol 57: 18, 2015.
28. Lin J and Redies C: Histological evidence: Housekeeping genes beta-actin and GAPDH are of limited value for normalization of gene expression. Dev Genes Evol 222: 369-376, 2012.
29. Bach DH, Park HJ and Lee SK: The dual role of bone morphogenetic proteins in cancer. Mol Ther Oncolytics 8: 1-13, 2017.
30. Ro TB, Holt RU, Brenne AT, Hjorth-Hansen H, Waage A, Hjertner O, Sundan A and Borset M: Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23: 3024-3032, 2004.