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SAGE analysis highlights the importance of *p53csv*, *ddx5*, *mapkapk2* and *ranbp2* to multiple myeloma tumorigenesis ☆

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ABSTRACT

Serial analysis of gene expression (SAGE) allows a comprehensive profiling of gene expression within a given tissue and also an assessment of transcript abundance. We generated SAGE libraries from normal and neoplastic plasma cells to identify genes differentially expressed in multiple myeloma (MM). Normal plasma cells were obtained from palatine tonsils and MM SAGE library was generated from bone marrow plasma cells of MM patients. We obtained 29,918 SAGE tags from normal and 10,340 tags from tumor libraries. Computer-generated genomic analysis identified 46 upregulated genes in the MM library. Ten upregulated genes were selected for further investigation. Differential expression was validated by quantitative real-time PCR in purified plasma cells of 31 patients and three controls. *P53CSV, DDX5, MAPKAPK2* and *RANBP2* were found to be upregulated in at least 50% of the MM cases tested. All of them were also found upregulated in MM when compared to normal plasma cells in a meta-analysis using ONCOMINE microarray database. Antibodies specific to DDX5, RANBP2 and MAPKAPK2 were used in a TMA containing 57 MM cases and confirmed the expression of these proteins in 74%, 96%, and 21% of the MM samples, respectively. Analysis of differential expression using SAGE could identify genes important for myeloma tumorigenesis (*P53CSV*, *DDX5*, *MAPKPK2* and *RANBP2*) and that could potentially be useful as therapeutic targets.

1. Introduction

Multiple myeloma (MM) remains an incurable malignant neoplasm, characterized by monoclonal proliferation of plasma cells in the bone marrow, serum or urinary monoclonal protein, osteolytic lesions, anemia and hypercalcemia [1]. High-dose chemotherapy followed by autologous stem cell transplantation increases complete response rates and overall survival. However, few patients, if any, will be cured. Allogeneic stem cell transplant can represent a chance of cure for selected cases but implies in high mortality and morbidity [2]. Currently, antiangiogenic drugs, such as thalidomide and its analogs (lenalidomide), and proteasome inhibitors (bortezomib) have been used as single or combined agents for relapsed/refractory

☆ A study from the clinical genomics project in multiple myeloma, Brazil

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cases and even as first line therapy [3,4]. Thus, despite of all these new therapeutic alternatives, MM remains an incurable disease, with median survival of 3 years [1]. Considering the heterogeneity of genetic aspects related with MM pathogenesis, recent studies have been focused on prognostic factors that could define the best therapeutic approach for each case or to contribute for the development of new therapies [5,6]. In this way, gene expression profiling analysis can be very helpful to define such prognostic factors. Serial analysis of gene expression (SAGE) allows the identification of genes expressed in a given tissue and also the assessment of their expression levels. However, the most important advantage of using SAGE is the possibility of undertaking a gene expression profiling without prior knowledge of the presence and sequence of genes to be analyzed, as opposed to microarrays [7]. Therefore, the aim of this study was to use SAGE to profile gene expression of normal and neoplastic plasma cells.

2. Material and methods

2.1. Patients and controls

Between June 2002 and April 2006, we obtained purified CD138-positive MM cells using CD138 (Sydecan-1) Micro Beads selection by Manual Ability Classification System (MACS) magnetic cell sorter (Miltenyi Biotec Inc., CA, USA) from 31 newly diagnosed MM patients, referred to the Hematology and Hemotherapy Service of Federal University of São Paulo, UNIFESP, São Paulo, Brazil. MM diagnosis was based on The International Working Group Criteria [8] and information on tumor stage was obtained for all patients according to Durie Salmon and the International Staging System (ISS) [9]. Only patients with no previous chemotherapy, corticosteroids or bisphosphonates treatment were included. Patients were treated with conventional chemotherapy (VAD or melphalan/prednisone or thalidomide/prednisone). Seven patients were consolidated with autologous bone marrow transplantation. Three pools of normal plasma cells were obtained from palatine tonsils (from seven children who underwent tonsillectomy). They were used as source of normal plasma cells because the number of these reactive cells is higher in tonsils than in normal bone marrow samples (only 1–5% of plasma cells), mainly after using sorting of CD138-positive cells. Written informed consent was obtained from all patients and controls, and the study was approved by the Ethical Committee of our institution. The main clinical characteristics of all patients are summarized in Table 1. The neoplastic plasma cells used in the SAGE library construction were obtained from bone marrow of two IgGκ newly diagnosed MM patients who were diagnosed at stage IIIA, with International Scoring System (ISS) 2, being one 50 years-old male and one 60 years-old fe male. One of them had hypodiploid and the other had hyperdiploid karyotype. Both cases had no chromosome 13 deletion. For the SAGE normal library we used purified CD138+ cells (normal plasma cells) obtained from palatine tonsils (Fig. 1).

Table 1
Clinical characteristics of 31 MM patients used in RQ-PCR validation.

Clinical characteristics	Number	Frequency (%)
Age median: 62 (27–80) years		
Sex		
Female	10	32.3
Male	21	67.7
Isotype		
IgG	16	51.6
IgA	9	29.0
Light chain	6	19.4
Durie and Salmon stage		
IA	0	0
IIA	1	3.2
IIIA	19	61.3
IIIB	11	35.5
ISS		
1	2	6.5
2	10	32.3
3	17	54.7
NA	2	6.5

ISS = international staging system.
NA = not available.

2.2. Magnetic sorting of CD-138-positive cells

Purified normal and neoplastic plasma cells were obtained after magnetic sorting of CD-138-positive (syndecan-1) cells using MACS (Magnetic Cell Sorting of Human Cells, Miltenyi Biotec Inc., CA, USA) system, given at least 85% of purity, confirmed by flow citometry assays.

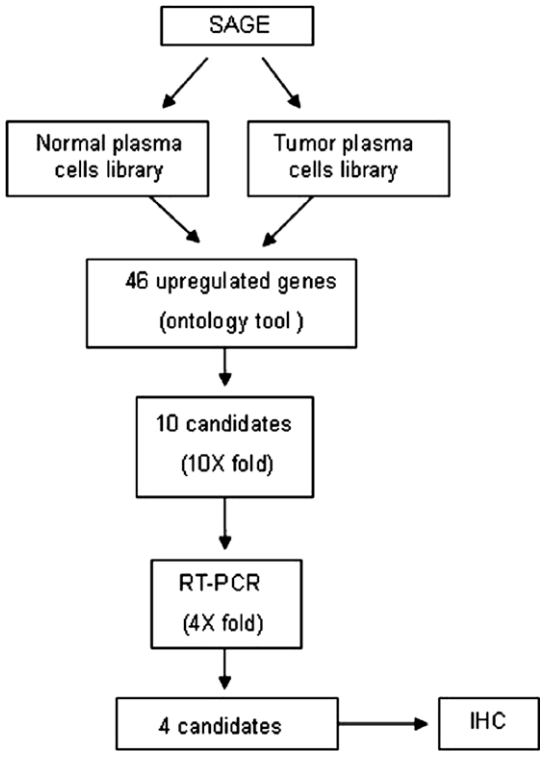

Fig. 1. Summary of study design.

2.3. RNA extraction and SAGE libraries construction

RNA extraction was performed using TRIzol reagent (Invitrogen, Carslbad, CA, USA) following the manufacturer's instructions. RNA quality was checked in 2% agarose gel stained with ethidium bromide. SAGE libraries were constructed using the I-SAGE kit (Invitrogen, Carlsbad, CA, USA), according to manufacture's instructions.

2.4. Sequencing of SAGE clones

Sequencing reactions were done on plasmid templates (pZErO-1 – Invitrogen, Carlsbad, CA, USA) using M13 Forward primer (5′-CGCCAGGTTTTCCCAGTCACGAC-3′) and the ABI Prism BigDye Terminator Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequencing reaction products were analyzed on 3700 ABI sequencer (Applied Biosystems, Foster City, CA, USA).

2.5. Genes selected for study

For further investigation, we selected genes that had tags that were unique and upregulated at least 10 times in the MM library as compared with the normal plasma cell library.

2.6. Quantitative PCR

For real-time PCR analyses, total RNA was also extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Two micrograms of RNA were used for first-strand cDNA synthesis with Superscript II and primed with oligo (dT) (Invitrogen, Carlsbad, CA, USA). After cDNA synthesis, the quality of cDNA preparation was evaluated by amplification of 311-bp *NOTCH2* fragment and agarose gel electrophoresis. Gene expression levels were evaluated by real-time PCR on ABI PRISM® 7500 Sequence Detection System Instrument (Applied Biosystems, Foster City, CA, USA). Primers and probes were obtained from the TaqMan Gene Expression Inventoried Assays (Applied Biosystems). *GAPDH* gene was chosen as the endogenous control based on previous publications [10,11]. TaqMan Universal PCR Master Mix (Applied Biosystems) was used to perform real-time PCR reactions and all the samples were analyzed in triplicate. Blank and positive controls were run in parallel to verify amplification fidelity. The relative mRNA expression level of the target genes was calculated using the $2^{-\Delta\Delta CT}$ method [12]. Genes were considered differentially expressed in tumor samples when their expression levels showed at least a 4-fold increment or decrease in comparison to normal samples.

2.7. Meta-analysis of microarray data from ONCOMINE™ database

We queried the ONCOMINE database (http://www.oncomine.org) for the expression level of the ten selected upregulated genes (at least 10 times in the MM library as compared with the normal plasma cell library) in five myeloma cancer studies deposited as of February 2008 [13–17], comparing (1) normal plasma cells and multiple myeloma samples; (2) monoclonal gammopaties of undetermined significance (MGUS) or smoldering myeloma with normal plasma cells; (3) monoclonal gammopaties of undetermined significance (MGUS) or smoldering myeloma and myeloma samples; (4) all studies comparing different clinical or cytogenetic categories within myeloma cases. ONCOMINE™ expression correlations were searched for each gene. Statistical analysis of differences was performed using ONCOMINE algorithms as previously described [18].

2.8. Immunohistochemistry analyses

We analyzed in duplicate the expression of DDX5, RANBP2, MAPKAPK2 in 57 confirmed MM cases using tissue microarray (TMA) method. Forty-seven samples were obtained from plasmacytomas of patients with MM diagnosis and 10 samples were obtained from bone marrow of MM patients. This cohort was completely independent (clinical data not shown) from the group of patients in which quantitative real-time PCR validation was performed. DDX5 was detected with the rabbit polyclonal anti-DDX5 (ab21696 – Abcam Inc., Cambridge, MA) at 1:12,000 and presented a nuclear localization. Rabbit monoclonal anti-MAPKAPK2 (ab51018 – Abcam Inc., Cambridge, MA) presented nuclear and cytoplasm expression pattern (titer 1:250). RANBP2 was detected with a rabbit polyclonal antibody (ab2938 – Abcam Inc., Cambridge, MA) and also presented nuclear and cytoplasm expression (titer 1:600). Antigen retrieval was done by Pascal Pressure Chamber. The visualization of primary antibody staining was performed using streptavidin-biotin-peroxidase method. The analyses were performed using ACIS®III Tissue Microarray Analysis (Dako – CA, USA). Nuclear staining was analyzed quantitatively from 0% to 100% (final results expressed by mean of two identical TMA slides). Cytoplasm staining intensity was scored from 1 to 255 (mean of two identical TMA slides) (Fig. 3). P53CSV protein expression was not validated because there is no antibody commercially available until this moment.

2.9. Statistical analysis

Associations between the variables (age, sex, isotype, Durie and Salmon stage, ISS, ploidy and del 13) were tested by the Pearson Chi-square test (X^2). Mann–Whitney test was used to perform mean comparisons. Differences with a $p < 0.05$ were determined to be statistically significant. Student *t*-test was used for analyzing the gene expression data sets from ONCOMINE database [18]. For ONCOMINE analyses, differences with a $p < 0.01$ were determined to be statistically significant.

3. Results

Normal plasma cells and MM libraries were obtained from 18 μg and 20 μg of total RNA, respectively. We generated 4992 clones from normal library and 3840 clones from MM library. These clones were submitted to automatic sequencing and we were able to acquire 29,918 tags from normal and 10,340 tags from tumor libraries. Using computer-generated genomic analysis tools (http://gdm.fmrp.usp.br/staff.php), we performed

the evaluation of data from both libraries. After clusterization, we obtained 12,913 unique tags from normal plasma cells library and 3595 unique tags from MM library. From those, 8241 and 2359 unique tags, respectively, matched genes presented in the UniGene databank (http://www.ncbi.nlm.nih.gov/entrez/query) (Table 2).

To select the differentially expressed genes, we established a high threshold of at least 10-fold to increase the discrimination power between normal and abnormal gene expression in plasma cells. This analysis allowed the identification of 46 upregulated genes and six down regulated genes in MM library (Tables 3 and 4). A further analysis of the functional classes of genes upregulated in the MM library using the gene ontology tool (http://www.geneontology.org/) revealed that most of them are involved in cell metabolism, cell structure and transcription processes. We decided to concentrate our analysis on the upregulated genes, because they could represent potential molecular therapeutic targets.

Ten candidate genes were selected to have their expression tested by real-time PCR in MM samples: *ZFHX1B*, *XBP1*, *PIM2*, *LGALS1*, *RANBP2*, *P53CSV*, *DDX5*, *LSM5*, *MAPKAPK2* and *SP140*. These genes were selected because they are involved in transcription regulation, signaling, cell proliferation and apoptosis, and therefore can have important roles in the tumorigenic process.

Comparative analysis of relative expression ($2^{-\Delta\Delta CT}$) of the 10 genes was undertaken in 31 MM cases and three controls (Fig. 2 and Table 5). Six genes (*P53CSV, DDX5, MAPKAPK2, RANBP2, LSM5* and *LGALS1*) were found to be significantly upregulated in the MM cases compared to the controls. From these genes, *P53CSV, DDX5, RANBP2* and *MAPKAPK2* were upregulated in at least 50% of cases tested (Table 5). We did not find any significant association between the expression level of the 10 genes evaluated and clinic pathological parameters of MM patients including age, sex, isotype, Durie Salmon stage and ISS.

To further validate our findings, we analyzed the expression profile of the 10 selected genes in microarray data deposited in ONCOMINE Database. From all MM studies available in this database, we selected the ones in which, similarly to our study, comparison between normal plasma cells and MM was undertaken [17,13]. *LGALS1, PIM2* and *ZHFX1B* were not found differentially expressed in the selected studies, but, we were able to confirm the over expression of four genes (*P53CSV, DDX5, MAPKAPK2* and *RANBP2*) in MM samples as compared to normal plasma cells (Table 6), which were the genes we identified using the combination of SAGE and real-time PCR analyses. Importantly, the meta-analysis of microarray data allowed the characterization of clinical situations in which these genes are upregulated (Table 6). We found that *P53CSV* is significantly upregulated early in disease development (MGUS) and in situations with predominantly unfavorable prognosis: MGUS comparing to normal bone marrow [13], smoldering MM comparing to normal bone marrow (similarly to our SAGE results) [13], plasma cell leukemia compared to MGUS [14], stage III comparing to stage I [15], more than four copies of 1q21 [15]. ONCOMINE meta-analysis showed that *DDX5* is upregulated in MM compared to normal bone marrow [5], similarly to our SAGE study. We also found that *DDX5* is upregulated in MGUS compared to both plasma cell leukemia and MM. *MAPKAPK2* in ONCOMINE was also found to be upregulated early in disease development (MGUS) and in situations with predominately unfavorable prognosis: MGUS comparing to normal bone marrow [13], smoldering MM comparing to normal bone marrow [13], MM comparing to normal bone marrow (in agreement with our SAGE study) [5], MGUS comparing to smoldering MM [13]. According to the same microarray studies, *RANBP2* seems to be upregulated early in disease development (MGUS) and in situations with predominately unfavorable prognosis: MGUS comparing to normal bone marrow [13], MM comparing to normal bone marrow [5], (in agreement with our SAGE results), smoldering MM comparing to normal bone marrow [13], stage III comparing to stage I [15], non-hyper diploid comparing to hyperdiploid karyotype [16].

Antibodies specific to three of our selected candidates (DDX5, RANBP2 and MAPKAPK2) were commercially available and were used on a TMA containing 57 MM samples. Nuclear positivity was detected in at least 50% of tumor plasma cells in 74% and in 96% of MM cases for DDX5 and RANBP2, respectively. Twenty-one percent of the MM cases showed over expression of MAPKAPK2. Intensity of cytoplasm staining varied from 81 to 177 for RANBP2 (median = 115.5) and from 51 to 121 (median = 63.5) for MAPKAPK2.

Table 2
Number of clones and unique tags generated by SAGE libraries.

	Normal plasma cells	Multiple myeloma
Sequenced clones	4992	3840
Total tags	29,918	10,340
Unique tags	12,913	3595
UniGene match	8241	2359
UniGene non-match	4438	1097

4. Discussion

The approach used in this study that comprised the combination of the SAGE methodology with real-time PCR validation, meta-analysis of microarray data and immunohistochemistry analyses allowed the identification of genes that are upregulated early in MM development and in situations of unfavorable prognosis. Therefore these genes may be involved in tumorigenesis process and may constitute therapeutic targets for this disease.

Gene expression patterns can provide important clues to the nature of molecular alterations that lead to cancer. SAGE data generated by CGAP, or deposited by other investigators, can be accessed through CGAP website (http://www.ncbi.nlm.nih.gov/SAGEmap) [19]. So far, there were no data of gene expression in normal or neoplastic plasma cells in public SAGE databases, which justifies the present study in where we compared normal and neoplastic plasma cells.

Since normal bone marrow usually has less than 5% of reactive plasma cells, purified CD138-positive cells obtained from palatine tonsils seemed to be a better source of normal RNA to perform gene expression studies. Zhan et al. [20] compared the gene expression pattern of MM plasma cells (MM1–MM4) and three types of normal counterparts (tonsil B-cells, tonsil plasma cells, bone marrow plasma cells). They showed that MM4 aggressive subtype is a tonsil B-cell-like MM. MM3 subgroup was strongly linked to tonsil plasma cells and MM2 subgroup was more related to bone marrow plasma cells. Their conclusions were that future studies would attempt to link each MM subtype to a normal cell counterpart. According to this, the use of tonsil plasma cells as a control source for SAGE and quantitative real-time PCR analyses was justified because in our study the majority of the cases analyzed were advanced MM.

All samples used in the study were obtained at MM diagnosis, i.e., before administration of any treatment (including corticosteroids), avoiding interference of treatment in gene expression results. To obtain enough RNA for construction of the MM SAGE library, we combined bone marrow plasma cells from two IgGκ newly diagnosed MM patients. We believe that since IgGκ is the most common MM isotype (~60% of cases), the results obtained after expression analyses could be relevant for the majority of cases and could be valid for the other isotypes as well.

Overexpression of *P53CSV* (or *TRIAP1* – TP53 regulated inhibitor of apoptosis 1) was found in 90% of our MM cases. *P53CSV* is one of the important players in P53-mediated cell survival pathway and is significantly induced when

Table 3
Characteristics of the 46 upregulated genes in MM library as compared to the normal plasma cells library.

ID gene	*Gene symbol*	Gene name	Function	Ratio (tumor/normal)
NM 0000990	*RPL2A*	Hypothetical protein	–	58
NM 0145270	*LOC146325*	Hypothetical protein	–	39
NM 1001794	*MGC33692*	Hypothetical protein	–	34
NM 0006476	*ATP5L*	ATP synthase	Cell metabolism	32
NM 0014795	*ZFHX1B*	Zinc finger homeobox 1b	Transcription, cell metabolism	32
NM 0003115	*UAP1*	UDP-N-acetylglicosamine	Cell metabolism	28
Hs 525646	*IGHG3*	Immunoglobulin heavy constant gama	–	24
NM 005080	*XBP1*	X box binding protein 1	Immune response, transcription	21
NM 017584	*ALDRL6*	Aldehyde reductase like 6	Signaling, cell metabolism	21
NM 003295	*TPT1*	Tumor protein, translationally-controlled 1	Cell metabolism	20
NM 001959	*EEF1B2*	Eukaryotic translation elongation factor 1 beta 2	Cell metabolism	19
NM 006875	*PIM2*	Pim 2 oncogene	Transcription, signaling, cell metabolism, proliferation	17
NM 002305	*LGALS1*	Lectin, galactoside-binding soluble 1 (galectin 1)	Signaling, apoptosis, proliferation	17
NM 153702	*ELMOD2*	ELMO domain containing 2	Apoptosis, cell structure	17
NM 004048	*B2M*	Beta-2-microglobulin	Immune response	17
NM 144646	*IGJ*	Immunoglobulin J polypeptide	–	16
NM 006267	*RANBP2*	Ran binding protein 2	Transcription	14
NM 173614	*NOMO2*	NODAL modulator 2 isoform 2	Cell structure, cell metabolism	14
NM 001689	*ATP5G3*	ATP synthase, H+ transporting	Cell structure, cell metabolism	14
NM 007220	*CA5B*	Inactivation escape 2	Cell metabolism	14
NM 016399	*P53CSV*	P53 inducible cell-survival factor	Cell cycle control, apoptosis	14
NM 203431	*PPIA*	Peptidylprolyl isomerase A isoform 2	Immune response	14
NM 015866	*PRDM2*	PR domain containing 2	Transcription	13
NM 205835	*LISCH7*	Liver-specific bHLH-Zip transcription factor isoform 3	Cell structure	12
Hs 531856	*GAS5*	Growth arrest-specific 5	Cell cycle control	12
NM 198440	*DERL3*	Der1-like domain family, member 3 isoform a	Cell structure	12
NM 024818	*UBE1DC1*	Ubiquitin-activating enzyme E1 domain containing 1 isoform 1	Cell metabolism	11
NM 004396	*DDX5*	DEAD (Asp–Glu–Ala–Asp) box polypeptide 5	Transcription	10
NM 001614	*ACTG1*	Actin gamma 1	Cell structure	10
NM 177947	*ARMCX3*	Armadillo repeat containing, X linked 3	Cell cycle control	10
NM 013339	*ALG6*	Asparagine-linked glycosylation 6	Cell structure, cell metabolism	10
NM 024068	*MGC2731*	Hypothetical protein	–	10
NM 012322	*LSM5*	LSM5 homolog u6 small nuclear RNA associated	Transcription	10
NM005354	*JUND*	Jun D proto-oncogene	Transcription, signaling, apoptosis	10
NM 022909	*CENPH*	Centromere protein H	Cell structure	10
NM 015914	*TXNDC11*	Thioredoxin domain containing 11	Cell metabolism	10
NM 003133	*SRP9*	Signal recognition particle 9 kDa	Cell metabolism	10
NM 001402	*EEF1A1*	Eukaryotic translation elongation factor 1 alpha 1	Cell metabolism	10
NM 004759	*MAPKAPK2*	Mitogen-activated protein kinase 2	Signaling	10
NM 1005176	*SP140*	SP140 nuclear body protein isoform 1	Immune response, transcription	10
NM 004374	*COX6C*	Cytochrome c oxidase subunit Vic	Cell metabolism	10
NM 016270	*KLF2*	Kruppel like factor 2 (lung)	Transcription	10
NM 004822	*NTN-1*	Nitrin 1	Signaling, apoptosis	10
NM 002101	*GYPC*	Glycophorin C isoform 1	Cell structure, cell metabolism	10
NM 005917	*MDH1*	Malate dehydrogenase 1, NAD	Cell metabolism	10
NM 021210	*TRAPPC1*	Trafficking protein particle complex 1	Cell metabolism	10

Table 4
Downregulated genes in the MM library compared to the normal plasma cells library.

ID gene	Gene symbol	Gene name	Function	Ratio (tumor/normal)
NM 018268	*UBE2G2*	Ubiquitin-conjugating enzyme E2G2	Cell metabolism	−11
Hs 449585	*IGLC2*	Immunoglobulin lambda variable 3–21	Cell adhesion	−11
NM 002038	*CLDN2*	Claudin 2	Cell adhesion and structure	−14
NM 001911	*HLA-DRA*	Major histocompatibility complex, class II, DR alpha	Immune response	−14
Hs 549046	*IGKC*	Immunoglobulin kappa constant	Immune response	−14
NM 001475	*PHYHIP*	Phytanoyl-CoA hydroxylase interacting protein	Protein binding	−14

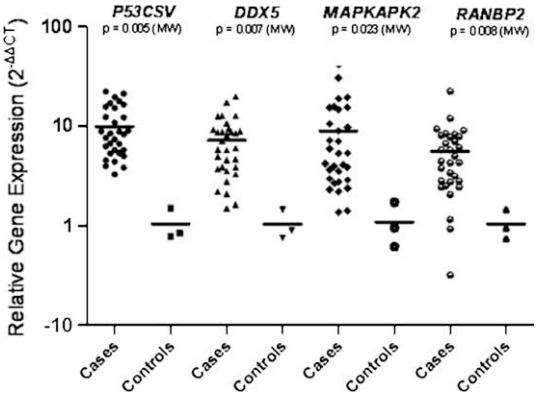

Fig. 2. Relative expression ($2^{-\Delta\Delta CT}$) of the validated genes.

cells are submitted to low levels of genotoxic stress. Thus, this protein prevents induction of apoptosis [21]. *P53CSV* can modulate apoptotic pathways through interaction with heat-shock protein 70 (Hsp70) that probably inhibits activity of apoptosis protease activating factor 1. Furthermore, inhibition of another heat-shock protein, Hsp90, is one of the promising strategies for MM control [22]. Therefore, our results suggest that the P53CSV/Hsp70 pathway should be evaluated as a new potential target for MM treatment.

DDX5, upregulated in MM, encodes a DEAD box protein, which is a RNA-dependent ATPase, and also a proliferation-associated nuclear antigen, specifically reacting with the simian virus 40 tumor antigens. DEAD box proteins are implicated in a number of cellular processes involving alteration of RNA secondary structure, such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly. Based on their distribution patterns, some members of this family are believed to be involved in embryogenesis, spermatogenesis, and cellular growth and division [23]. As demonstrated by Zhan et al. [17] *DDX5* is upregulated in MM in relation to normal bone marrow. The same was detected by our gene expression analyses (upregulated in 67% of MM cases) but Mattiolli et al. [14] showed some contradictory data. According to their results *DDX5* is significantly upregulated in MGUS compared to MM plus plasma cell leukemia. Our IHC analysis showed that DDX5 was also upregulated in 74% of MM cases examined.

MAPKAPK2 (MAPK [p38 mitogen-activated protein kinase] mitogen-activated protein kinase-2) encodes a member of the Ser/Thr protein kinase family and was found upregulated in 58% of the MM cases investigated. This kinase is regulated through direct phosphorylation by p38 MAP kinase. In conjunction with p38 MAP kinase, this protein is known to be involved in many cellular processes including stress and inflammatory responses, nuclear export, gene expression regulation and cell proliferation. One of the major substrates of MAPKAPK2 is the heat-shock protein Hsp27, which stimulates actin polymerization in order to facilitate recovery from destruction of cytoskeleton during cellular stresses. Hideshima et al. [24] have shown that overexpression of Hsp27 confers resistance to

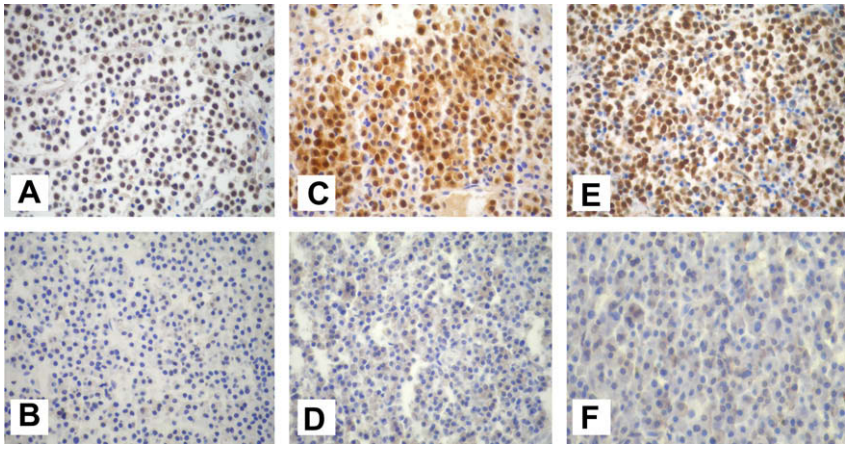

Fig. 3. Panel showing MM cases: (A) positive for DDX5; (B) negative for DDX5; (C) positive for MAPKAPK2; (D) negative for MAPKAPK2; (E) positive for RANBP2 and (F) negative for RANBP2 (400×).

Table 5
Chromosomal localization and real-time PCR analyses of gene expression.

Gene	Upregulation (%)	Downregulation (%)	Controls		Cases		*p* Value*
			Median	Range	Median	Range	
P53CSV	90.3	0	1.09	1.00–1.93	8.31	3.28–22.23	0.005
DDX5	67.7	0	1.19	1.00–1.91	6.00	1.49–19.91	0.007
XBP1	64.5	0	2.78	1.00–3.96	5.05	0.59–29.83	0.95
MAPKAPK2	58.1	0	1.54	1.00–2.77	5.35	1.38–41.51	0.023
RANBP2	58.1	0	0.42	0.27–1.00	4.45	0.32–22.40	0.008
LSM5	45.2	0	1.13	1.00–1.30	3.25	0.95–11–15	0.019
LGALS1	32.3	0	0.40	0.38–1.00	2.68	0.39–25.48	0.010
SP140	25.8	3.2	1.93	1.03–3.50	2.23	0.21–10.05	0.649
PIM2	25.8	3.2	2.04	1.00–2.38	2.04	0.11–12.28	0.761
ZFHX1B	6.4	67.8	0.83	0.31–1.00	0.67	0.04–9.48	0.738

* Mann–Whitney test = comparison between gene expression in tumor and normal plasma cells.

Table 6
Meta-analysis of microarray data available at ONCOMINE Database.

Gene	Study	Observation (number of patients)	*p* Value	Reporter	Reference
P53CSV	Zhan_Myeloma_3	Upregulated in MGUS (early stage) (44) vs. normal bone marrow (normal) (22)	1.5E−5	218403_at	13
	Zhan_Myeloma_3	Upregulated in smoldering multiple myeloma (12) vs. normal bone marrow (normal) (22)	5.5E−6	218403_at	13
	Mattioli_Myeloma	Upregulated in plasma cell leukemia (late stage) (6) vs. MGUS (early stage) (7) and multiple myeloma (39)	0.001	218403_at	14
	Agnelli_Myeloma_2	Upregulated in stage III 34) vs. stage I (30)	1.9E−4	218403_at	15
	Carrasco_Myeloma	Upregulated in 1q21 amplification 4+ (8) vs. 2 (27)	4.1E−4	218403_at	16
DDX5	Zhan_Myeloma	Upregulated in multiple myeloma (74) vs normal B-cell – tonsil (7), normal plasma cell – bone marrow (30), normal plasma cell – tonsil (normal)(7)	0.004	X15729_s_at	17
	Mattioli_Myeloma	Upregulated in MGUS (early stage) (7) vs. multiple myeloma (39) and plasma cell leukemia (late stage) (6)	5E−4	200033_at	14
MAPKAPK2	Zhan_Myeloma_3	Upregulated in MGUS (early stage) (44) vs. normal bone marrow (normal)(22)	1.4E−4	201460_at	13
	Zhan_Myeloma	Upregulated in multiple myeloma (74) vs. normal – B-cell – tonsil (7), plasma cell – bone marrow (30), plasma cell – tonsil (normal) (7)	7.8E−6	X75346_s_at	17
	Zhan_Myeloma_3	Upregulated in smoldering multiple myeloma (12) vs. normal bone marrow (normal) (22)	3.6E−5	201460_at	13
	Zhan_Myeloma_3	Upregulated in smoldering multiple myeloma (12) vs. MGUS (early stage) (44)	0.002	215050_x_at	13
RANBP2	Zhan_Myeloma_3	Upregulated in MGUS (early stage) (44) vs. normal bone marrow (normal) (22)	1.3E−4	226922_at	13
	Zhan_Myeloma	Upregulated in multiple myeloma (74) vs. normal B-cell – tonsil (7), plasma cell – bone marrow (30), plasma cell – tonsil (normal)(7)	7.7E−4	D42063_at	17
	Zhan_Myeloma_3	Upregulated in smoldering multiple myeloma (12) vs. normal bone marrow (normal) (22)	0.001	201713_s_at	13
	Agnelli_Myeloma_2	Upregulated in stage III (34) vs. stage I (30)	0.008	201713_s_at	15
	Carrasco_Myeloma	Upregulated in non-hyperdiploid (28) vs. hyperdiploid (37)	0.002	201713_s_at	17

Reporter: microarray probe set identification; numbers between parenthesis: refer to number of analyzed cases in the respective studies.

bortezomib. Therefore, overexpression of *MAPKAPK2* could be related to MM resistance to chemotherapy and/or pathogenesis. They hypothesized that inhibition of *MAPKAPK2* activity could augment bortezomib cytotoxicity by downregulating Hsp27. Our gene expression studies also support further exploitation of this pathway as therapeutic target in MM, although immunohistochemistry did not show high frequency of protein expression (only 21% of cases presented more than 50% of nuclear positivity) in an independent cohort of MM samples.

RANBP2 (*RAN* binding protein-2) belongs to the complex of proteins related to RAN, a member of the *Ras* family of GTPase proteins that has a role in many aspects of cell biology, including shuttling of protein and RNA in and out of the nucleus as well as regulating chromosome condensation, spindle formation, nuclear assembly, and cell-cycle progression. The high frequency of RANBP2 nuclear expression in our MM cases (96%) suggests the importance of this gene in MM biology. Therefore, all the attempts to inhibit RANBP2 overexpression could be useful in MM control.

The merit of the present study is to mine four genes previously included in arrays studies but which importance was not explored until now in MM. Analysis of a larger number of patients is necessary to confirm these findings and to ascertain if *P53CSV*, *DDX5*, *MAPKAPK2* and *RANBP2* should be further explored as potential new biomarkers or therapeutic targets for MM treatment.

Conflict of interest statement

None declared.

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