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ORIGINAL ARTICLE

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Highly expressed genes in multiple myeloma cells – what can they tell us about the disease?

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Abstract

Cancer cells can convert proto-oncoproteins into oncoproteins by increasing the expression of genes that are oncogenic when expressed at high levels. Such genes can promote oncogenesis without being mutated. To find overexpressed genes in cancer cells from patients with multiple myeloma, we retrieved mRNA expression data from the CoMMpass database and ranked genes by their expression levels. We grouped the most highly expressed genes based on a set of criteria and we discuss the role a selection of them can play in the disease pathophysiology. The list was highly concordant with a similar list based on mRNA expression data from the PADIMAC study. Many well-known "myeloma genes" such as MCL1, CXCR4, TNFRSF17, SDC1, SLAMF7, PTP4A3, and XBP1 were identified as highly expressed, and we believe that hitherto unrecognized key players in myeloma pathogenesis are also enriched on the list. Highly expressed genes in malignant plasma cells that were absent or expressed at only a low level in healthy plasma cells included IFI6, IFITM1, PTP4A3, SIK1, ALDOA, ATP5MF, ATP5ME, and PSMB4. The ambition of this article is not to validate the role of each gene but to serve as a guide for studies aiming at identifying promising treatment targets.

KEYWORDS
B2M, BCMA, CD74, FOS, HBB, JUN, multiple myeloma, TCTP, TPT1, β2-microglobulin

1 | INTRODUCTION

Multiple myeloma (MM) is a cancer of plasma cells, the terminal differentiation stage of B lymphocytes. The cancer cells, MM cells, are usually located in the bone marrow (BM) where their harmful influence regularly leads to bone degradation[1] and often to anemia, hypercalcemia, and renal dysfunction.[2] Although recurrent genetic aberrations have been identified in MM cells, none of the aberrations are ubiquitous and none of them have proved useful as drug targets in treatment regimens. On the other hand, effective treatment is established against molecules that are not aberrant but which are highly expressed by the cancer cells like CD38, SLAMF7, and BCMA.[2] In this article, we have employed mRNA expression data from CoMMpass, a database established by the Multiple Myeloma Research Foundation,

Novelty statement
The paper ranks genes in primary myeloma cells according to expression level. A high proportion of the genes are "well-known myeloma genes", but many genes on the list have never been studied by myeloma researchers despite playing an important biological role and despite carrying significant prognostic information.

and identified highly expressed genes with the presumption that proteins encoded by such genes might be suitable as treatment targets.

2 | MATERIAL AND METHODS

Gene expression data from 864 samples of purified and RNA-sequenced myeloma cells (MM cells) derived from 767 patients in the CoMMpass database (IA11 release) were downloaded. We calculated the average expression of the individual genes across all samples and selected the 300 protein-coding nuclear genes with the highest expression levels. Genes coding for proteins involved in the translation of mRNA (n = 108) were deleted from the list, leaving 192 genes for further analysis.

Genes were classified according to five properties. First, they were sorted by the function of their encoded protein. We defined 20 functional groups specifically for this publication and assigned each gene to one of these groups after having studied literature about the corresponding protein. Next, they were dichotomized into yes or no for the following four properties: (1) whether the gene was a "known MM gene", (2) whether the gene was expressed by all patients or only by a subgroup, (3) whether expression level conferred prognostic information, and (4) whether the gene was classified in the Depmap database[3,4] as a gene that MM cells are dependent on for survival. A flowchart of the gene sorting is shown in Figure 1. "Known MM gene" was loosely defined as being a main focus in at least one MM-related English-language paper in PubMed. The criterion for being expressed only by a subgroup was that the gene was silent [defined as expression level <30 fragments per kilobase million (FPKM)] in >5% of patients and that there was a ratio of >1000 between the highest and the lowest expression level of the gene.

Survival data available from CoMMpass were used to assess the prognostic impact of the genes. For each gene, the patients were dichotomized into groups of equal size as high or low expressors based on whether their expression of the gene was over or below the median expression for the whole group of patients. The Kaplan-Meier method was used for survival analyses, and the survival curves were compared with the log-rank test. A gene was defined as prognostic if the hazard ratio for overall survival for either high or low expressors was >1,35 with a p-value <.01. The software available on the CoMMpass website was used to identify genes with prognostic relevance according to this criterion. GraphPad Prism 8 was used for creating the survival curves and the statistical analysis presented in Figure 2.

3 | RESULTS

3.1 | Highly expressed genes

The 300 most highly expressed protein-coding genes in primary MM cells were listed and ranked in descending order by expression level. Genes coding for ribosomal proteins or for other proteins involved in mRNA translation were highly abundant among the first couple of hundred mRNAs on the list. This may reflect the housekeeping nature of many of the proteins encoded by these genes, but such genes may also be more highly expressed in MM cells than in other cells due to the vast production of immunoglobulin. Whether correct or not, we thought such housekeeping proteins might be considered of little relevance to oncogenesis. Based on this decision, 79 of the first one hundred and 29 of the next two hundred mRNAs were deleted. The remaining 192 mRNAs were analyzed further (Table 1 shows the first 50 genes on the list and Table S1 the whole list). To verify this list, we downloaded similar mRNA expression data from MM cells sampled from 44 MM patients enrolled in the PADIMAC study.[5] Of the 50 genes with the highest expression in the CoMMpass data, 45 were also present on the PADIMAC list (Table 1). In total there was 63% (120/192) concordance between the two lists (Table S1). The concordance between the CoMMpass list and a list of expression data from normal BM plasma cells[6] was 41 genes among the 50 highest expressed in CoMMpass (Table 1) and 54% (103/192) in total (Table S1).

The highest expressed gene was B2M, coding for β2-microglobulin. The third was TPT1, encoding translationally controlled tumor protein (TCTP). This protein is an anti-apoptotic molecule that interacts directly with and stabilizes Mcl-1, a BCL2 family molecule.[7] MCL1 itself was number 12 on the list of highly expressed genes, second of molecules classified as anti-apoptotic. TPT1 and MCL1 were ranked as number 78 and 96 on the list of mRNA expression in normal plasma cells (Table 1), indicating an increased role in malignant plasma cells.

3.2 | Known "myeloma genes" and genes expressed by only a subgroup of patients

First, we identified genes that were already known to be implicated in MM pathogenesis. A conspicuously high number of genes [52 (27%)] coded for proteins that had previously been described as playing a role in MM. Among them were genes coding for the proteins β2-microglobulin,[8] Mcl-1,[9] CXCR4,[10] Cyclin-D1,[11] Syndecan-1,[12] SLAMF7,[13] Serglycin,[14] Pim2,[15] BCMA,[16] PRL-3,[17,18] and several members of the AP1 family of transcription factors (c-Jun,[19] JunB,[20] and c-Fos[21]) (Table 1). "Known MM genes" were more often prognostic (42%) than genes that have not been described in the context of MM (36%). However, this difference was not statistically significant (chi-squared test: 0,48).

Further, we assessed whether each gene was ubiquitously expressed or expressed only by a subgroup of patients. Merely 15 of the 192 genes were expressed by only a subgroup. Interestingly, 11 out of the 15 mRNAs (73%) with skewed expression were coding for proteins previously described as playing a role in MM, a much higher percentage than in the group of ubiquitously expressed mRNAs (23%) (chi-squared test: <0,001).

Despite that the molecules expressed by a subgroup were more often mentioned in the literature on MM, they were not significantly

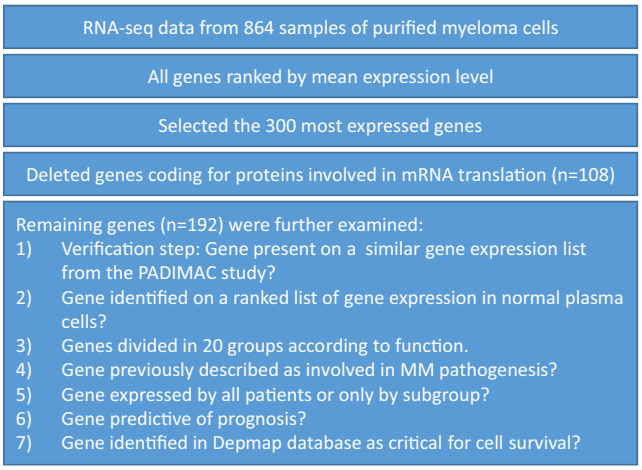

FIGURE 1 Flowchart of data analysis

more often prognostic (40%) than the ubiquitously expressed mRNAs (67 out of 177, (38%)).

3.3 | Genes classified by function

Next, we grouped genes according to known function. The group "Regulation of metabolism" comprised 26 genes, and all were ubiquitously expressed (Table 2), probably reflecting the housekeeping function of many of these genes. Only three of them (11.5%) were classified as "known MM genes". Nevertheless, 46% of the metabolism genes were of prognostic significance. Six genes in this group coded for proteins in the mitochondrial ATP synthase complex and four genes coded for proteins in the terminal enzyme of the mitochondrial respiratory chain, cytochrome c oxidase, probably reflecting a highly active respiratory chain in MM cells. COX8A, the gene coding for cytochrome c oxidase subunit 8A, was strongly associated with a bad prognosis (Figure 2). Four of the respiratory chain genes had much lower expression levels in normal plasma cells. They were COX8A and three genes coding for proteins in the F0 subunit of the ATP synthase complex, ATP5ME, ATP5MF, and ATP5MG (Table S1). Three glycolysis enzyme genes (LDHA, GAPDH, and ALDOA) were also on the list. LDHA had very strong prognostic impact (Figure 2), whereas ALDOA had very low or absent expression in normal plasma cells.

Another large group (26 members) were genes encoding proteins involved in protein processing, typically enzymes that catalyze posttranslational modifications. With one exception (CST3), all these genes were ubiquitously expressed. Here, only three genes (11.5%) were "known MM genes". Of the mRNAs in this group, 54% carried significant prognostic information, as compared to 36% of the genes not belonging to this group (chi-squared test: 0,074). In all instances, high expression of the gene implied an unfavorable prognosis.

Four genes in the protein processing group (OST4, DAD1, DDOST, and STT3a) were parts of the oligosaccharyltransferase complex. This complex is involved in the N-linked glycosylation of proteins in the endoplasmic reticulum (ER) by transferring an oligosaccharide to asparagine residues.

The third largest group, "function in ER", consisted of 22 genes. The proteins encoded by these genes typically serve as chaperons in proteins folding or are involved in protein trafficking in ER, Golgi, or secretory vesicles. Three of the four "known MM genes" in this group were heat shock proteins. An astonishing 62% of the genes were prognostic, as compared to 35% in genes not belonging to this group (chi-squared test: 0,030). Like in the previous group, all genes were ubiquitously expressed.

The next group, called "signal transduction", contained 19 genes. Well-known members here were the signaling receptors SLAMF7,[13] BCMA,[16] and CXCR4.[10] Other members were enzymes in signaling pathways like RHOB and the phosphatase genes DUSP1, DUSP5, and PTP4A3.[22] SIK1 and PTP4A3 were highly overexpressed in malignant plasma cells as they ranked only as number 12557 and 8155, respectively, in normal plasma cells (Table S1). Ten of these genes were "known MM genes", a much higher percentage than in the previous groups. Surprisingly, only 26% of the genes in this group were prognostic. Nevertheless, the expression of SRGN, the gene encoding serglycin, stood out as being highly significant for overall survival (Figure 2). Serglycin is the most abundant proteoglycan in MM cells and has been linked to MM-promoting processes including cell growth, cell adhesion, bone resorption, angiogenesis, and complement inhibition.[14,23,24]

Twelve genes coded for transcription factors. Here eight genes (67%) were "known MM genes", but none of the 12 mRNAs conferred any prognostic information. Five transcription factors were members of the AP-1 family, JUN, JUNB, JUND, FOS, and FOSB. Three of them were "known MM genes" (JUN,[19] JUNB,[20] and FOS[21]). FOS, JUN, and FOSB were ranked number 10, 14, and 20 of all genes on the list. Only XBP1, coding for the transcription factor X-box binding protein 1, which is essential for differentiation of plasma cells,[25] was expressed at the same level (number 13). XBP1 mRNA exists in both un-spliced variants (XBP1u) and a spliced variant (XBP1s), the latter coding for a larger protein isoform than XBP1u. XBP1s is of particular interest since it is the isoform that can initiate the so-called unfolded protein response (UPR).[26] The CoMMpass database also contains expression data on mRNA isoforms and the ratio between XBP1u and XBP1s isoforms was 12.4. Only one of the 12 transcription factors (IER2) was absent on the gene list of MM samples from the PADIMAC study, whereas 10 of them ranked lower in expression in normal plasma cells than in MM cells (Table 1 and Table S1).

Genes being mostly involved in immune regulation also contained 12 entries. Three of them (25%) were expressed by a subgroup only, two of which encode the interferon-inducible proteins IFI6 and IFITM1. IFI6 and IFITM1 ranked only as number 2621 and 5205, respectively, in normal BM plasma cells. Four genes were of prognostic relevance and two of them, PTMA[27] and MIF,[28] were particularly strong negative prognostic factors (Figure 2).

Nine genes coded for cytoskeletal proteins or proteins regulating the cytoskeleton. VIM and ACTB, coding for vimentin and β-actin, respectively, ranked number 19 and 23 on the overall list of highly expressed genes. High levels of MYL6 and CFL1, encoding myosin light chain-6 and cofilin-1, respectively, were particularly strong negative prognostic factors (Figure 2).

Seven genes coded for proteins involved in protein degradation. They were all ubiquitously expressed. Only PSMB4 was a "known MM gene", encoding proteasome subunit beta 4, one of the 17 essential subunits of the 20S proteasome complex and the subunit that regulates assembly of the proteasome.[29] In the CoMMpass data set, PSMB4 expression was a highly significant prognostic factor (Figure 2), and it was the only gene in the protein degradation group to carry prognostic information. PSMB4 was overexpressed in MM cells as its expression ranked number 2714 in normal BM plasma cells (Table S1).

Six genes were coding for metal chelators, three of them calcium-binding (S100A6, S100A8, and S100A9) and two ferritin subunits (FTL and FTH1). Surprisingly, HBB, the gene coding for the beta chain of hemoglobin, was one of the genes in this group, and ranked number 72 on the list of highly expressed genes in MM cells. Four of the genes in this group (66%), including HBB, were expressed by only a subgroup of patients, the highest fraction in any of the functional groups.

Three molecules classified as adhesion molecules were on the list, SDC1, ITGB7, and LGALS1, the genes coding for syndecan-1,[12] integrin beta-7,[30] and galectin-1,[31] respectively. All three were "known MM genes". ITGB7 and LGALS1 were of prognostic significance, whereas SDC1 was not.

3.4 | Cross search to identify genes critical for cell survival

To explore whether any of the highly expressed genes could represent a unique vulnerability in MM cells, we did a cross search between our list of highly expressed genes and a list from the online Depmap database consisting of genes enriched in MM from either CRISPR- or RNAi-based screens.[3,4,32] Of the 192 highly expressed genes in the CoMMpass dataset, 21 were revealed as critical for survival of MM cells in the Depmap screens (Tables 1 and 2). Twelve (57%) of the 21 vulnerability genes were "known MM genes", a much higher fraction than in genes not reported in the Depmap screens (7%) (chi-squared test: 0,001). B2M, MCL1, XBP1, PIM2, and PTP4A3 were among these 12 genes.

4 | DISCUSSION

The list of highly expressed genes in the CoMMpass data set had a very high fraction (27%) of molecules with a described role in MM. This suggested to us that the other genes also might be enriched by molecules of high relevance to the pathogenesis in MM. We therefore consider the list to be of value as a reference to researchers working on MM. A similar list from an independent data set (PADIMAC) showed high concordance and served as verification of the data.

We found that 38% of the genes predicted disease outcome. Despite rarely being studied by MM researchers, genes coding for proteins engaged in "protein processing" or with a "function in ER", had a very high fraction of prognostic mRNAs (56% combined). High levels of such proteins might reflect high cellular activity, rapid growth, and cell division. In addition, MM cells have high production of proteins for export, resulting in many misfolded proteins. Therefore, the unfolded protein response (UPR) could be important in MM cells.[33] Some of the gene products in these gene groups are involved in the UPR. Expression of UPR genes could be induced by XBP1s, the spliced variant of the transcription factor XBP1, which was ranked number 13 in expression level.[26,33] XBP1s causes development of an MM-like disease when forcibly expressed in a mouse model, suggesting that XBP1s could play a role as a driver of MM pathogenesis.[34] However, in the CoMMpass data, XBP1s was expressed at lower levels than the un-spliced XBP1 variants.

Members of the oligosaccharyltransferase complex constitute a cluster of genes in the protein processing group that could possibly become targets for the treatment of MM. Genes encoding the catalytic subunit, STT3a, and three other members of this complex (OST4, DAD1, DDOST) were highly expressed. All of them were prognostic, but none have been studied in the context of MM.[35,36] DAD1 protein, also called Defender of Apoptotic cell Death, is reported to bind Mcl-1 and protect against apoptosis.[37]

The group of transcription factors, comprising 12 different mRNAs, were at the other extreme with regard to clinical impact, as none of them were prognostic. Intuitively, this is difficult to understand since these genes code for proteins that regulate molecular programs supposedly important for the aggressiveness of cancer. Posttranscriptional regulation that leads to low correlation between mRNA level and protein level could be a possible explanation.

Genes expressed by only a subgroup of patients were more often "known MM genes" than ubiquitously expressed genes. It could be that genes with a dynamic expression more easily will be interpreted as important and more often studied than molecules with a stable expression pattern. An exaggerated focus on molecules with skewed expression is supported by the modest prognostic impact of such molecules.

The most highly expressed of all 192 genes was B2M, encoding β2-microglobulin, the beta moiety of the HLA class I heterodimer and a well-known prognostic serum marker in MM.[38] Besides its role in antigen presentation, it also delivers a "don't eat me" signal to macrophages,[39] which could be an important protective measure for cancer cells against the immune system. Contrary to the β2-microglobulin serum level,[40] B2M mRNA was not prognostic. Maybe the level in serum is not primarily reflecting gene expression but cancer cell turnover. Aggressive, highly proliferating disease could be accompanied by rapid cell decay, which may lead to release of

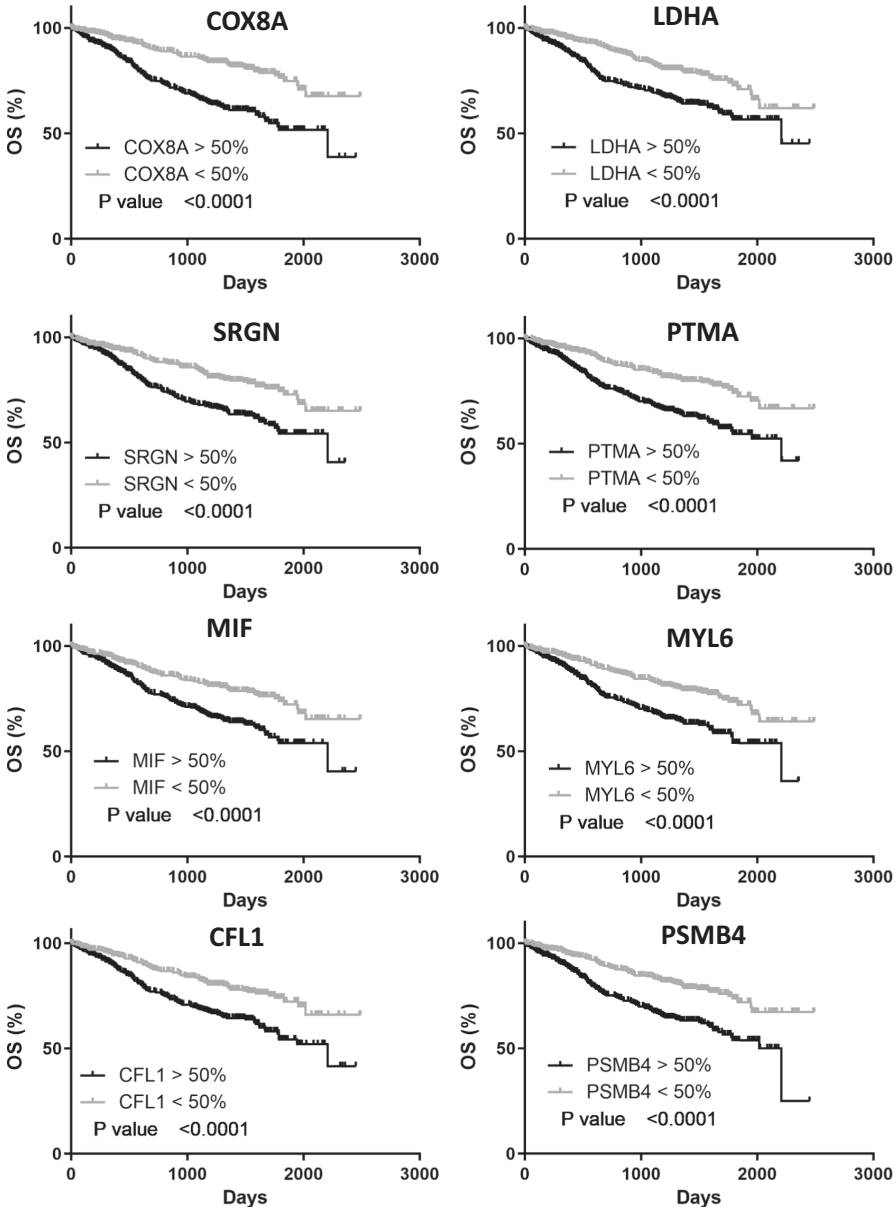

FIGURE 2 Genes with expression highly correlated with patient survival. Kaplan-Meier plots showing overall survival of patients with high or low expression of genes as specified in the figure. Data from the CoMMpass data bank, IA15. The survival curves were compared with the log-rank test. The statistical analyses were performed in GraphPad Prism

β2-microglobulin into circulation. The alternate explanation that β2-microglobulin in serum reflects tumor load, is less likely since percentage of MM cells in the BM is not among the best measures of disease aggressiveness.[41]

Several studies have pointed to Mcl-1 as the most important anti-apoptotic molecule of the BCL-2 family in MM cells.[9,42] Interestingly, MCL1 ranked number 12 of highly expressed genes and was the only BCL-2 family molecule on the list.

The only other anti-apoptotic gene on the list, TPT1, encoding translationally controlled tumor protein (TCTP), was mentioned in passing in a single paper on MM.[43] The lack of interest in TPT1 is surprising given that it was the third most expressed gene and that it is reported to stabilize Mcl-1.[7,44] Notably, expression of both these anti-apoptotic genes ranked higher in malignant than in normal plasma cells.

Twelve mRNAs coded for immunoregulatory proteins. Two of them, IFITM1 and IFI6, encoded proteins involved in interferon signaling. A gene set enrichment analysis (GSEA) comparing primary patient cells with MM cell lines revealed that immune signaling signatures are significantly enriched in primary cells, and interferon response genes are an important part of this enrichment.[45] We have found that induction of interferon response genes is mediated by transcription factors STAT1 and −2 in response to PRL-3 (protein encoded by PTP4A3) in MM cells.[46] It is not unlikely that cellular traits that are exclusive to primary cells reflect the influence of the microenvironment in the BM. IL-6 and other cytokines that induce important signaling mediators such as PRL-3 could be instrumental in sustaining important molecular programs that are not necessarily operative in cell lines.[47,48] It is also highly noteworthy that expression of IFI6, IFITM1, and PTP4A3 was very low in normal BM plasma cells, showing that their expression in plasma cells is virtually exclusive to malignant plasma cells.

MIF, coding for macrophage migration inhibitory factor, is a secreted cytokine and a ligand for two receptors encoded by genes on the list, CD74 and CXCR4. This opens for the possibility of autocrine

TABLE 1 List of highly expressed genes in samples of purified primary myeloma cells, ranged by expression level

Number	GENE name	Mean of gene expression (FPKM)	Gene classification	Known myeloma gene?	Prognostic?	Ubiquitously expressed (U) or by subgroup (S)	Among top 300 genes in the Padimac study	Expression rank in normal BM PCs	Enriched in MM in Depmap
1	B2M	19959	Antigene presentation	Y	N	U	Y	1	Y
2	IGJ	4294	Immune regulation	N	N	U	Y	2	N
3	TPT1	4227	Anti-apoptosis	N	N	U	Y	78	N
4	HLA-B	3399	Antigene presentation	Y	N	U	Y	6	N
5	TXNDC5	3322	Function in ER	Y	N	U	N	1631	Y
6	HLA-C	2825	Antigene presentation	N	N	U	Y	15	N
7	FTL	2557	Metal chelation	N	N	U	Y	12	N
8	MZB1	2289	Immune regulation	N	N	U	Y	9	N
9	HSP90B1	1556	Function in ER	Y	Y	U	Y	4	Y
10	FOS	1550	Transcr factor	Y	N	U	Y	19	N
11	DDX5	1335	Transcr regul	Y	N	U	Y	18	N
12	MCL1	1333	Anti-apoptosis	Y	Y	U	Y	96	Y
13	XBP1	1308	Transcr factor	Y	N	U	Y	8	Y
14	JUN	1291	Transcr factor	Y	N	U	Y	31	N
15	TSC22D3	1268	Immune regulation	N	Y	U	Y	23	N
16	CYBA	1184	Immune regulation	N	N	U	Y	41	N
17	DERL3	1176	Function in ER	N	N	U	Y	852	N
18	NACA	1130	Function in ER	N	N	U	Y	376	N
19	VIM	1092	Cytoskeleton	Y	N	U	Y	177	N
20	FOSB	1088	Transcr factor	N	N	U	Y	169	N
21	CD74	1083	Antigene presentation	Y	N	U	Y	5	N
22	PPIB	1070	Function in ER	N	Y	U	Y	144	N
23	ACTB	1025	Cytoskeleton	N	N	U	Y	3	N
24	HLA-A	1024	Antigene presentation	Y	N	U	Y	29	Y
25	H3F3B	1007	Histon	N	N	U	Y	14	N
26	HSPA8	983	Function in ER	N	N	U	Y	36	N
27	DUSP1	928	Signal transduction	N	N	U	Y	17	N

TABLE 1 (Continued)

Number	GENE name	Mean of gene expression (FPKM)	Gene classification	Known myeloma gene?	Prognostic?	Ubiquitously expressed (U) or by subgroup (S)	Among top 300 genes in the Padimac study	Expression rank in normal BM PCs	Enriched in MM in Depmap
28	HNRNPH1	914	RNA processing	N	N	U	Y	295	N
29	TMSB10	872	Cytoskeleton	N	Y	U	Y	43	N
30	ITM2C	871	Unknown	Y	N	U	Y	25	N
31	PPIA	805	Immune regulation	N	Y	U	Y	201	N
32	UBC	797	Prot degrad.	N	N	U	Y	11	N
33	COX7C	794	Metabolism	N	N	U	N	581	N
34	JunD	745	Transcr factor	N	N	U	Y	371	N
35	HSPA5	734	Function in ER	Y	Y	U	Y	7	Y
36	HNRNPA1	734	RNA processing	N	N	U	Y	903	N
37	GAPDH	721	Metabolism	N	Y	U	Y	45	N
38	OAZ1	716	Metabolism	N	Y	U	Y	77	Y
39	SPCS1	716	Function in ER	N	Y	U	N	67	N
40	ATF4	715	Transcr factor	Y	N	U	Y	158	N
41	PSAP	699	Metabolism	N	N	U	Y	16	N
42	H1FX	683	Histon	N	N	U	Y	519	N
43	GNAS	682	Signal transduction	N	N	U	Y	192	N
44	SEC61B	681	Function in ER	N	Y	U	N	191	N
45	TMBIM6	681	Function in ER	N	Y	U	Y	28	N
46	ACTG1	671	Cytoskeleton	N	N	U	Y	13	N
47	TMSB4X	647	Cytoskeleton	Y	N	S	N	21	N
48	NFKBIA	639	Signal transduction	Y	N	U	Y	56	N
49	TRIB1	631	Signal transduction	N	N	U	Y	76	N
50	CCNL1	623	Cell division	N	N	U	Y	167	N

Abbreviations: FPKM, fragments per kilobase million; N, no; Y, yes; PCs, plasma cells; S, expressed by a subgroup; U, expressed by all patients.

TABLE 2 Groups of highly expressed genes in samples of purified primary myeloma cells, categorized by function of encoded protein

Gene groups defined by function of encoded protein	Number of genes	Fraction of "known MM genes" (%)	Fraction of prognostic mRNAs (%)	Fraction of genes expressed by subgroup (%)	Fraction of genes in top 192 genes in normal PCs (%)	Fraction of genes vulnerable by Depmap (%)
Regulation of metabolism	26	11.5	46	0	59.1	3.8
Protein processing	26	11.5	53.8	3.8	58	3.8
Function in ER	22	18	59	0	63.6	13.6
Signal transduction	19	52.6	26.3	10.5	63	15.8
RNA processing	14	0	42.9	0	43	0
Transcription factor	12	66.7	0	16.7	50	8.3
Immune regulation	12	33.3	33.3	25	41.7	8.3
Transcriptional regulation	10	20	30	0	40	30
Cytoskeleton	9	33.3	44.4	11.1	88.9	11.1
Protein degradation	7	14.3	14.3	0	43	14.3
Antigene presentation	6	83	0	0	100	33
Metal chelation	6	33	66	66	17	16.7
Unknown	5	20	20	0	40	20
Ion transport	4	25	50	0	75	0
Cell division	4	25	0	25	25	25
Adhesion	3	100	66.7	33.3	0	0
Anti-apoptosis	2	50	50	0	100	50
Histon	2	0	0	0	50	0
Mitochondrial transporter	2	0	0	0	0	0
Protein trafficking, not ER	1	0	100	0	100	0
All genes	192	27.1	38	7.8	54	10.9

stimulation. It is shown that MIF is involved in adhesion of MM cells to BM stroma and in resistance to chemotherapeutic drugs.[28] Its importance for MM cells is further supported by the strong correlation between MIF expression and a bad prognosis.

The high expression of HBB was surprising. Not much is written about the role of hemoglobin proteins in cancer. A recent publication showed the HBB gene product β-globin to be expressed by breast cancer cells in circulation, but not by primary or metastatic tumor cells.[49] The function of the protein was not primarily to carry oxygen, but to lower ROS levels.

PSMB4 has not been extensively studied in MM, but a publication from 2015 found this molecule to be overexpressed in MM cells and its expression to be positively correlated with NFκB activity and expression of miRNA21.[29] PSMB4 is also overexpressed and linked to adverse prognosis in several cancer entities and could be a cancer-driving gene in solid tumors.[50] Mutations and overexpression of another subunit, PSMB5, are determinants for resistance to the proteasome inhibitor bortezomib,[51] but whether PSMB4 overexpression can lead to resistance to proteasome inhibitors is an open question that needs to be examined.

Surprisingly, the expression of SDC1, encoding syndecan-1, lacked prognostic significance. Syndecan-1 serum level is a powerful negative prognostic factor.[41] The explanation could be the same as suggested above for B2 M. Alternatively, it could be that syndecan-1 is shed from the cell surface through an active enzymatic process[52] and that soluble syndecan-1 has oncogenic properties.[53,54]

In conclusion, we think that the list of highly expressed genes in MM cells both confirms the importance of molecules that already have been extensively studied and points at molecules that deserve further examination.

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CONFLICT OF INTEREST

The authors have no conflicts of interest relevant for this paper.

DATA AVAILABILITY STATEMENT

All the data processed in this article were retrieved from public sources. CoMMpass data can be accessed on this website: https://research.themmrf.org. The gene-dependencies enriched in MM cells can be accessed through depmap.org/portal/context/multiple_myeloma. Publicly available mRNA sequencing data from MM cells sampled from 44 patients in the PADIMAC study[5] and mRNA sequencing data from 31 samples of normal BM plasma cells from healthy donors[6] can be downloaded from GEO, accession numbers GSE116324 and GSE178788, respectively. These two lists of gene expression levels were ranked and filtered in the same way as the CoMMpass data.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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