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Genome-wide CRISPR/Cas9 screen identifies regulators of BCMA expression on multiple myeloma cells

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Multiple myeloma (MM) is a blood malignancy defined by an uncontrolled clonal growth of plasma cells. Currently, new immunotherapies are being introduced that utilize BCMA to redirect the patient's T-cells to kill MM cells, using either chimeric antigen receptor (CAR) T-cells [1] or bispecific BCMAxCD3 T-cell-engaging antibodies [2, 3]. These agents show unprecedented effects in early clinical trials, including an almost complete initial response rate in patients with refractory MM and durable remissions in ~50% of these patients [1–5]. However, the efficacy depends on the BCMA expression level. Downregulation of BCMA can limit long-term effectiveness and lead to relapse [6–8]. Conversely, cytokine release syndrome has been reported in patients with extremely high levels of BCMA expression on MM cells [9]. Hence, it is essential to understand the regulation of BCMA expression.

BCMA, encoded by *TNFRSF17*, is a receptor surface glycoprotein receptor expressed on plasma cells [10, 11]. Previously, only a few regulators of BCMA expression have been reported. Firstly, the γ-secretase protease cleaves BCMA and about 140 other transmembrane proteins [12], including amyloid precursor protein. γ-secretase inhibitors, originally developed to prevent amyloid formation in Alzheimer's disease [13], have been suggested as a means to boost BCMA-targeted immunotherapy for MM [14–16]. Secondly, the POU2AF1, PRDM1, IRF4, and RUNX3 transcription factors and the IL4 and IL6 cytokines have been suggested to upregulate BCMA [17–22].

To search for BCMA regulators, we conducted a genome-wide CRISPR/Cas9 screen in the OPM2 and MOLP8 MM cell lines using the Brunello library [23], containing 76,441 small guide RNA (sgRNA) sequences targeting 19,114 genes. We isolated BCMA-high-expressing ($BCMA^{hi}$) and BCMA-low-expressing ($BCMA^{lo}$) cells by fluorescence-activated cell sorting and assessed sgRNA representation using massively parallel sequencing. The details of the experiments are described in Supplementary Methods.

Twenty-six genes showed significant differences in sgRNA representation (Supplementary Table 1). These genes showed a strong correlation between OPM2 and MOLP8 effect sizes (Pearson $r = 0.70$, $P = 6.0 \times 10^{-5}$). We observed enrichment of *TNFRSF17* in $BCMA^{lo}$ cells (Fig. 1; Supplementary Table 1), confirming specificity. Analysis of bulk- and single-cell mRNA-sequencing data showed enrichment of expression of the 26 genes in plasma cells (Supplementary Figs. 1–3). To see if the identified genes regulate other MM immunotherapy targets, we carried out similar CRISPR/Cas9 screens for CD38 and CD319, observing no convincing effects for any of the 26 genes (Supplementary Table 2).

The identified set of genes showed more functional interactions than expected (STRING database [24]; 26 interactions *vs.* seven expected, $P = 3.4 \times 10^{-8}$), with γ-secretase and oligosaccharyl transferase genes forming distinct subnetworks (Supplementary Fig. 4; Supplementary Table 3). The most enriched gene in $BCMA^{hi}$ cells was *PSENEN*, encoding presenilin enhancer 2, an essential γ-secretase subunit [25, 26]. γ-secretase also contains nicastrin (NCSTN), presenilin 1 or 2 (PSEN1 or PSEN2), and aph1 homolog A or B (APH1A or APH1B). NCSTN is a substrate-recruiting component [27], PSEN1 and PSEN2 alternative active subunits [28], and APH1A and APH1B alternative stabilizing subunits [29–32]. Combinatorically, the incorporation of either APH1A or APH1B and either PSEN1 or PSEN2 produces four types of γ-secretase [31–38]. We found enrichment in $BCMA^{hi}$ cells of *NCSTN*, *PSEN1*, and *APH1A* but not for *PSEN2* and *APH1B* (Fig. 1, Supplementary Table 1, 2). Directed CRISPR/Cas9 knockdown of the four γ-secretase genes identified in the screen increased BCMA expression up to 11.2-fold, whereas knockdown of *APH1B* and *PSEN2* produced weaker effects (Fig. 2 and Supplementary Fig. 5).

In $BCMA^{lo}$ cells, we detected enrichment of 6 genes involved in protein N-glycosylation (*STT3A, DDOST, ALG5*, *TMEM258*, *RPN2*, and *OST4*; Fig. 1, Supplementary Table 1). BCMA was recently identified as a glycoprotein with a complex type N-glycan at a single N-glycosylation site, asparagine 42, and altered glycosylation affects BCMA ligand binding [39]. Strikingly, *DDOST*, *STT3A*, *RPN2*, *TMEM258*, and *OST4* all encode subunits of the oligosaccharyltransferase (OST) complex that catalyzes the initial transfer of high-mannose oligosaccharides (Glc(3)Man(9)GlcNAc(2)) to asparagine residues within the Asn-X-Ser/Thr motif, the first step in N-glycosylation [40]. *ALG5* encodes an enzyme required for the addition of glucose residues to the oligomannose core [41]. For further validation, we knocked down five of the N-glycosylation genes by directed CRISPR/Cas9, observing 3.6-fold downregulation of BCMA on average (Fig. 2 and Supplementary Fig. 5). These data indicate that N-glycosylation is required for BCMA presentation on the MM cell surface.

Somatic loss-of-function mutations in genes required for BCMA expression could confer resistance to BCMA-targeted immunotherapies. To understand if loss-of-function mutations in the identified N-glycosylation genes are tolerated by MM cells, we analyzed CRISPR/Cas9 knockdown effects in the DepMap compendium, observing no or only mild suppression of cell growth (median Chronos gene score >-1) for 5 of the 6 N-glycosylation genes (Supplementary Fig. 6). Consistent with this, none of the N-glycosylation genes showed evidence of intolerance to loss-of-function variants in the Genome Aggregation Database (Supplementary Table 4). Additionally, germline loss-of-function mutations in *DDOST* underlie Congenital Disorder of Glycosylation type Ir, an autosomal recessive disorder characterized by developmental defects, intellectual disability, and humoral immunodeficiency [42, 43]. Loss-of-function mutations in *ALG5* have been reported in atypical polycystic kidney disease. These observations suggest that loss-of-function mutations in the N-glycosylation genes are unlikely to lead to clonal elimination.

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In addition to γ-secretase genes and N-glycosylation genes, we detected 16 genes significantly affecting BCMA expression. For example, in BCMA^{hi} cells, we saw strong enrichment of *HEXIM1* (HEXIM P-TEFb Complex Subunit 1) and *UBE2M* (ubiquitin-conjugating enzyme E2M). *HEXIM1* functions as an RNA polymerase II inhibitor [44] and regulator of NF-κ-B and corticosteroid-driven transcription [45, 46], which play key roles in MM. *UBE2M* encodes an E2 ubiquitin ligase that attaches ubiquitin to proteins to trigger their degradation. Directed knockdown of *HEXIM1* and *UBE2M* upregulated BCMA 2.3-fold and 3.8-fold, respectively (Supplementary Fig. 5). Interestingly, no ubiquitination mechanism has been described before for BCMA. Additional genes of interest include those implicated in transcriptional regulation (*TP53TG3B*, *POLR1A*, *CNIH1*, *ZNF792*, *TCEB2*), mitochondrial metabolism (*TAZ*, *CO15*), and ribosome biogenesis (*SDAD1*, *LTV1*).

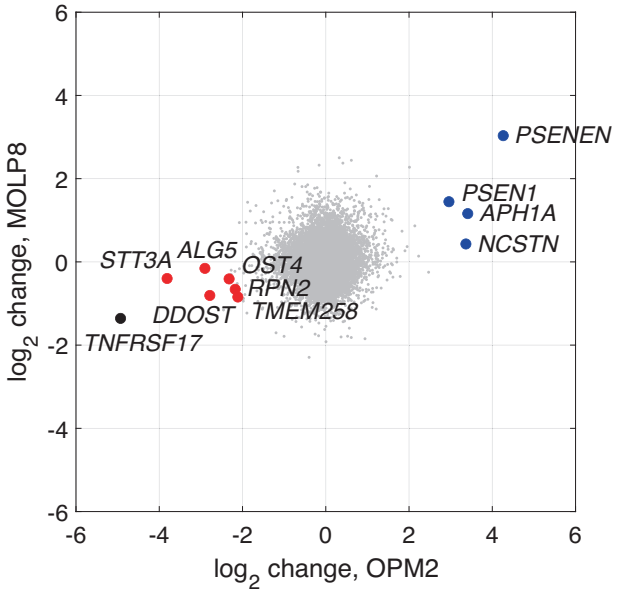

Fig. 1 Screening results. We performed genome-wide CRISPR/Cas9 screens in the OPM2 and MOLP8 MM cell lines. We sorted BCMA-high-expressing (BCMA^{hi}) and BCMA-low-expressing (BCMA^{lo}) cells, determined the sgRNA abundance by massively parallel sequencing, and calculated $\log_2$ ratios reflecting the sgRNA frequency in BCMA^{hi} relative to BCMA^{lo} cells. The *x* and *y* axes represent OPM2 and MOLP8 cells, respectively. *Blue*: Genes encoding γ-secretase subunits. *Red:* Genes encoding oligosaccharyltransferase subunits or other enzymes involved in N-glycosylation. *Black: TNFSRF17*, which encodes BCMA itself. The summary statistics are given in Supplementary Table 1.

In summary, we report a genome-wide screen for regulators of BCMA expression. Using conservative criteria, we identify 26 genes. Of these, only the four γ-secretase genes belong to a biological process previously implicated in BCMA regulation [14, 15]. These results have potential for clinical translation: Firstly, we confirm γ-secretase as a potent negative regulator of BCMA expression. In a recent phase-1 study, patients with relapsed MM were pre-treated with γ-secretase inhibitor before receiving BCMA CAR T-cells [16], producing an average 12.2-fold upregulation of BCMA, which is on par with our findings. Our data and this trial warrant intensified studies to determine the value of adding γ-secretase inhibitors to BCMA-directed immunotherapy. Secondly, we identify impaired N-glycosylation as a tentative resistance mechanism to BCMA-targeted immunotherapies. Accordingly, these genes should be investigated further in samples of MM patients resistant versus sensitive to BCMA-targeting immunotherapies; such data sets will likely become available once BCMA-targeting agents are used on a larger scale. Finally, we identify several new genes that could potentially be utilized to boost BCMA expression, including several additional regulators with strong effects (*e.g.*, *HEXIM1* and *UBE2M*). While detailed investigations of each of these genes are beyond the scope of this study, further studies should be performed to verify the mechanistic impact on the anti-MM activity of T-cell-engaging immunotherapies in both cell lines and primary MM cells. Our work provides new insight into the regulation of BCMA expression, with potential implications for the treatment of MM.

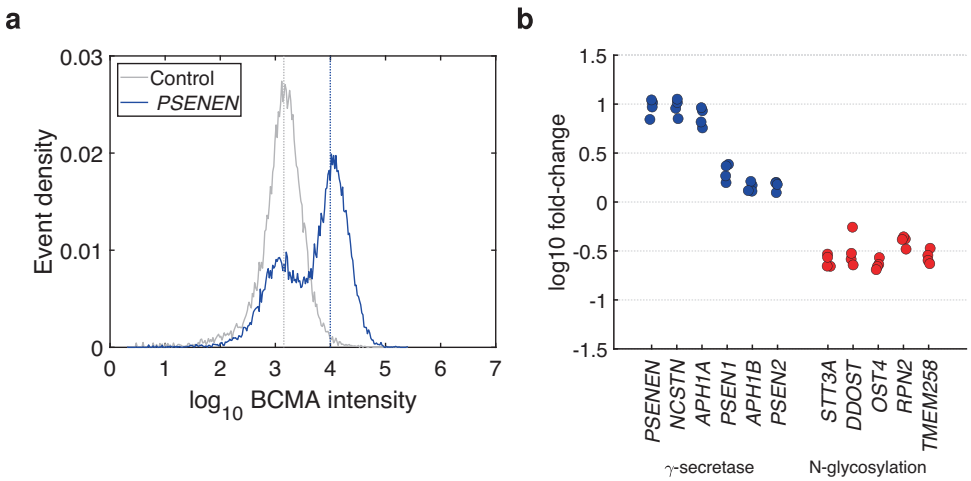

Fig. 2 Summary of validation data. To validate our screening results and directly estimate effects on BCMA expression, we performed CRISPR/Cas9 knockdown of 14 genes in OPM2 cells (detailed data in Supplementary Fig. 5). **a** Representative example showing the effects of CRISPR/Cas9 knockdown, in this case of the *PSENEN* gene. CRISPR/Cas9-treated cells show a bimodal distribution (blue), reflecting CRISPR-edited and unedited cells. Untreated cells show a unimodal distribution (grey). Using Gaussian Mixture Modeling, we estimated the mean BCMA intensity of the right-shifted cell population (blue line). We calculated the log10 fold-change relative to the mean intensity of untreated cells (grey line). **b** Summary of changes in BCMA expression for all genes tested x four biological replicates each. *Blue:* Genes encoding γ-secretase subunits. *Red:* Genes encoding oligosaccharyltransferase subunits or other enzymes involved in N-glycosylation.

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DATA AVAILABILITY

The raw sequencing data from our CRISPR/Cas9 screens in MOLP8 and OPM2 cells have been deposited in the Sequence Read Archive (SRA; accession number PRJNA1043457).

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AUTHOR CONTRIBUTIONS

RA and BN designed the project. RA carried out the experiments. JM contributed to the experiments. BN, RA, MP, LE, and ZA conducted the bioinformatic analysis. RA and BN drafted the manuscript. All authors contributed to the final manuscript.

COMPETING INTERESTS

JM is employed by BioInvent International AB. The remaining authors have no conflicts of interest to declare.

ADDITIONAL INFORMATION

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