Figures and figure supplements

Paradoxical resistance of multiple myeloma to proteasome inhibitors by decreased levels of 19S proteasomal subunits

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**Figure 1.** Screen for genes controlling the sensitivity of multiple myeloma cells to carfilzomib. (A) Screening strategy. (B) Gene Ontology (GO) categories enriched among the top 50 genes whose depletion results in sensitization to carfilzomib and the top 50 genes whose depletion results in desensitization to carfilzomib.

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**Figure 2.** Nodes within the proteostasis network control the response of myeloma cells to carfilzomib. (A) Volcano plot showing knockdown effects (sensitization or desensitization to carfilzomib) and statistical significance of human genes (orange dots) and quasi-genes generated from negative control shRNAs (grey dots). Drug resistance / sensitization phenotypes were previously defined as $\rho$ (Kampmann et al., 2013); a value of $-1$ corresponds to a twofold sensitization to the drug. Hit genes belonging to functional categories of interest are color-coded as labeled in the panels. (B) Volcano plot as in (A), except showing effect on growth. Growth phenotypes were previously defined as $\gamma$ (Kampmann et al., 2013); a value of $-1$ corresponds to a twofold reduction in growth rate. (C) Volcano plot as in (A), highlighting the opposing effects of 20S or 19S proteasome knockdown on the sensitivity of cells towards carfilzomib. Note the protective effect is not restricted to the 19S regulator alone, but is shared with the 11S regulator. (D) Volcano plot as in (C), except showing effect on growth.

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Figure 2—figure supplement 1. Comparison of growth phenotypes and carfilzomib resistance phenotypes for each targeted gene. Hit genes belonging to functional categories of interest are color-coded.
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Figure 3. Rapamycin desensitizes cells to carfilzomib. Dose-response curves of multiple myeloma (MM) cells (A, B) and a leukemia cell line (C) exposed to carfilzomib after a 24 hr pretreatment with 200 nM rapamycin. EC50: fold change of EC50. Data points are means of two experimental replicates, error bars denote SD. DOI: 10.7554/eLife.08153.006
Figure 3—figure supplement 1. Induction of autophagy desensitizes cells to carfilzomib. (A–C) Dose-response curves of MM cells (A, B) and a leukemia cell line (C) exposed to carfilzomib after a 24 hr pretreatment with 100 mM trehalose. FC: fold change of EC50. Data points are means of four experimental replicates, error bars denote SD. (D) The percent viable cells (compared to untreated) at the calculated EC50 for carfilzomib are interpolated from the dose response curves in (A–C) and Figure 3. p values are derived from a one-tailed Student’s t-test for unpaired samples. (E) Measurement of autophagy induction in U-266 cells transduced with a mCherry-EGFP-LC3B autophagic flux reporter and treated with 200 nM rapamycin or 100 mM trehalose for 24 hr. Reduction in GFP signal indicates an increase in autophagic flux. Chloroquine (75 μM, 24hr, co-incubation) was added as a control to show inhibition of autophagic flux.

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Figure 4. Opposing effects of 19S and 20S proteasomal subunit knockdown on carfilzomib sensitivity. (A, B) Scatter plots of the frequencies cells expressing different shRNAs targeting a 20S core subunit (A) or a 19S regulator subunit (B) in untreated or carfilzomib-treated cells. The grey dots represent cells expressing negative control shRNAs. Colored bars indicate the quantitative resistance phenotype (ρ) of each shRNA. (C) Heatmap showing the protective or sensitizing effect of knocking down subunits of the 19S or 20S proteasomes, respectively, in multiple cell lines. (D) Dose-response of U266 cells that constitutively expresses an shRNA targeting the PSMD12 subunit of the 19S proteasome, the PSMB5 subunit of the 20S proteasome, or a negative control shRNA. Data points are means of two experimental replicates, error bars denote SD.

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Figure 5. Proteasome activity in U266 cells expressing a negative control shRNA or an shRNA targeting the PSMD12 subunit of the 19S proteasome, and its susceptibility to inhibition by carfilzomib after a 1 hr treatment. (A) Fluorometric measurement of the chymotrypsin-like protease activity of the 20S proteasome. (B, C) Enzyme-linked immunoabsorbent assay for accessibility of the (B) β5 subunit and the (C) LMP7 subunits of the 20S proteasome. Data points are means of two experimental replicates, error bars denote SD. DOI: 10.7554/eLife.08153.009
Figure 5—figure supplement 1  Characterization of samples used to measure proteasome activity in Figure 5. (A) Immunoblot analysis of PSMD12 and PSMB5 levels. Numbers below the blots correspond to the normalized relative amount (compared to total protein in each lane). (B) PSMD12 transcript levels quantified by qPCR and normalized to GAPDH and to the untreated negative control cells. Data points are means of three primer pairs targeting the transcript, error bars denote SD. (C) Dose response to carfilzomib. Data points are means of two experimental replicates, error bars denote SD. DOI: 10.7554/eLife.08153.010
Figure 6. Depletion of the 19S protease regulator causes the accumulation of specific substrates. Immunoblot analysis of protein levels in U-266 cells expressing a negative control shRNA or an shRNA targeting 19S subunit PSMD12, untreated or exposed to low (200 nM) or moderate doses (2 μM) of carfilzomib for 4 hr. Numbers below the blots correspond to the normalized relative amount (compared to total protein in each lane). Numbers on the left Figure 6. continued on next page
Figure 6. Continued

The margin of each panel indicates molecular weights (kDa).

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Figure 6—figure supplement 1. Determination of the global effects of 19S proteasome depletion on the proteome. (A) Schematic of proteomics experiments in K562 cells expressing a negative control shRNA (N.C.) or an shRNA targeting the PSMD6 subunit of the 19S proteasome, either untreated or treated with bortezomib. (B) Log2 enrichment of proteins derived from proteome-wide SILAC in cells treated with bortezomib or in cells where the 19S proteasome subunit PSMD6 was knocked down. Overall Pearson correlation between the proteomic changes induced by the two treatments is 0.3. Blue and green lines correspond to the top 50 enriched proteins for each experiment. GO terms enriched within these sets were Protein Degradation (for PSMD6 knockdown, FDR-corrected p < 0.001) and Cell cycle (for bortezomib treatment, FDR-corrected p < 0.13) (C) Increased levels of factors involved in autophagy and the ubiquitin-proteasome system (UPS) specifically by PSMD6 knockdown. PSMD6 KD (bortezomib) refers to the effect of PSMD6 KD in the context of bortezomib treated samples (i.e. log2 ratio of PSMD6 KD + bortezomib over control KD + bortezomib), whereas Bortezomib (PSMD6 KD) refers to the effect of bortezomib treatment in the context of PSMD6 KD cells (i.e. the log2 ratio of PSMD6 KD + bortezomib over PSMD6 KD + DMSO). (D) Fold enrichment of ubiquitylated peptides caused by PSMD6 knockdown. Prominent hits represent the UPS and autophagy as protein degradation pathways. DOI: 10.7554/eLife.08153.012
Figure 7. 19S proteasomal subunit levels predict the response to carfilzomib-based therapy in patients. Levels of (A) 19S subunit PSMC2, (B) 20S subunit PSMB2 and (C) aggresomes quantified by flow cytometry in CD138 + bone marrow cells (including plasma cells and MM cells) of patients prior to therapy in clinical trial for carfilzomib-based combination therapy. Values are shown for separately for complete responders and partial responders.

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