Cancer Vulnerabilities Unveiled by Genomic Loss

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Cancer Vulnerabilities Unveiled by Genomic Loss

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SUMMARY

Due to genome instability, most cancers exhibit loss of regions containing tumor suppressor genes and collateral loss of other genes. To identify cancer-specific vulnerabilities that are the result of copy number losses, we performed integrated analyses of genome-wide copy number and RNAi profiles and identified 56 genes for which gene suppression specifically inhibited the proliferation of cells harboring partial copy number loss of that gene. These CYCLOPS (copy number alterations yielding cancer liabilities owing to partial loss) genes are enriched for spliceosome, proteasome, and ribosome components. One CYCLOPS gene, PSMC2, encodes an essential member of the 19S proteasome. Normal cells express excess PSMC2, which resides in a complex with PSMC1, PSMD2, and PSMD5 and acts as a reservoir protecting cells from PSMC2 suppression. Cells harboring partial PSMC2 copy number loss lack this complex and die after PSMC2 suppression. These observations define a distinct class of cancer-specific liabilities resulting from genome instability.

INTRODUCTION

Cancers arise as the result of the accumulation of somatic genetic alterations within a cell, including chromosome translocations, single base substitutions, and copy number alterations (Stratton et al., 2009). Although a subset of these alterations (“driver events”) promote malignant transformation by activating oncogenes or inactivating tumor suppressor genes, most somatic genetic alterations are the consequence of increased genomic instability that occurs in cancer but does not contribute to tumor development (“passenger events”).

The demonstration that cancers are often dependent on specific driver oncogenes has stimulated efforts to find and exploit these targets therapeutically. For example, cancers that harbor translocations that form fusion transcripts such as BCR-ABL or EML4-ALK or mutations such as EGFR or BRAF depend on the activity of these gene products for tumor maintenance (Brose et al., 2002; Daley et al., 1990; Soda et al., 2007). Therefore, the presence of such an alteration often predicts response to drugs that inhibit the function of these proteins (Sawyers, 2005).

An alternative strategy to target cancers is to target genes that are not oncogenes but are genes that cancers require to accommodate cancer-specific stress (Ashworth et al., 2011; Kaelin, 2005). In comparison to normal cells, cancer cells rely inordinately on pathways that abrogate a variety of cancer-related stressors that include DNA damage replication stress, proteotoxic stress, mitotic stress, metabolic stress, and oxidative stress (Solimini et al., 2007). Even though proteins within these pathways may be essential in all cells, genetic alterations may induce a state in which reliance on these pathways creates a therapeutic window as a result of cancer-specific stresses.

The proteasome, which recognizes and degrades proteins modified with a polyubiquitin chain (Finley, 2009), is one such target. Although proteasome function is essential to cells for basal protein turnover and for degradation of unfolded proteins, multiple myeloma cells produce excessive amounts of immunoglobulin and appear to be especially dependent on effective protein turnover by the 26S proteasome. Indeed, the 20S
proteasome inhibitor bortezomib is used as first-line treatment of multiple myeloma (Richardson et al., 2005).

Genomic instability may be another source of cancer-specific stress. The majority of human cancers harbor copy number alterations involving the loss or gain of broad chromosomal regions. For example, copy number losses that target tumor suppressor genes often involve multiple neighboring genes that may not contribute to cancer development. The loss of such neighboring genes has been postulated to render cancer cells highly vulnerable to further suppression or inhibition of those genes (Frei, 1993), but until recently, the tools to systematically test this hypothesis were not available. Here, we integrated both genome-scale copy number and loss-of-function data on a panel of 86 cancer cell lines to determine whether partial copy number loss of specific genes renders cells highly dependent on the remaining copy. We identified a class of genes enriched for cell-essential genes, most predominantly proteasome, spliceosome, and ribosome components, which render cells that harbor copy number loss highly dependent on the expression of the remaining copy.

RESULTS

Integration of Genome-Scale Copy Number and Gene Dependency Analyses Identifies CYCLOPS Genes

By analyzing copy number profiles from 3,131 cancers across a wide diversity of cancer types (Beroukhim et al., 2010), we found that most cancers exhibit copy number loss affecting at least 11% of the genome and that many cancers exhibit much more extensive loss of genetic material (Figure 1A). Much of this widespread genomic disruption is due to copy number alterations involving whole chromosomes or chromosome arms, presumably due to mechanisms that favor the generation of such large events (Figure 1B). As a consequence, most genes undergo copy number loss in a substantial fraction of cancers (average, 16.2; range, 3.7%–40.2%; Figure S1A available online). A subset of the genes affected by recurrent copy number losses contributes to cancer development as tumor suppressor genes; however, many genes are recurrently lost due to passenger events or because of their proximity to a frequently deleted tumor suppressor gene (Figures 1C and S1B). We hypothesized that, for a subset of nondriver genes, hemizygous loss may be tolerated and frequent, but complete loss would lead to cell death. In some of these cases, hemizygous loss might lead to sensitivity to further suppression of the gene relative to cells without copy number loss of these genes.

To identify genes whose loss correlated with a greater sensitivity to further gene suppression, we integrated gene dependencies and copy number data from 86 cancer cell lines (Table S1). We analyzed gene essentiality data from Project Achilles, a data set that scored the impact of individually expressing 54,020 short hairpin RNAs (shRNAs) targeting 11,194 genes on the proliferation of 102 cell lines (Cheung et al., 2011). For 7,250 of these genes, multiple shRNAs had comparable effects across cell lines, suggesting that their effects were due to suppression of the intended target. We used these shRNAs to construct composite “gene dependency scores” (A.T., W.C.H., and J.P.M., unpublished data). We also obtained DNA copy numbers for these same cell lines from Affymetrix SNP 6.0 array data (Bartlett et al., 2012). For each gene, we first classified each cell line by whether or not it exhibited copy number loss in that gene and then calculated the mean gene dependency score among cell lines in each class. We then determined the difference in mean scores between the copy-loss and copy-neutral classes and rated the significance of this difference by permuting class labels (Figure 1D). To minimize the confounding effect of lineage, all permutations maintained the initial lineage distribution within each class. We also restricted these analyses to the 5,312 genes for which each class contained at least seven cell lines. We identified 56 candidate genes with false discovery rate (FDR) (Benjamini and Hochberg, 1995) p values of less than 0.25 (Tables 1 and S2) and named them CYCLOPS (copy number alterations yielding cancer liabilities owing to partial loss) genes.

We validated the CYCLOPS vulnerabilities by using an independently generated RNA interference (RNAi) data set (shRNA Activity Rank Profile, shARP) (Marcotte et al., 2012) representing the consequences of expressing 78,432 shRNAs targeting 16,056 genes on the proliferation of 72 breast, ovarian, or pancreatic cancer cell lines. We applied the same analysis pipeline, which was constrained to the “validation set” of 47 cell lines for which we had copy number data and the 6,574 genes for which at least seven cell lines were in each class (copy loss and copy neutral) (Tables S1 and S2). These genes included 3,282 of the genes that underwent full analysis in the Achilles data set and 40 of the CYCLOPS candidates identified in that analysis. Although the lineage distribution was markedly different between the validation and Achilles data sets (breast and pancreatic cancers made up 90% of the cell lines in the validation set but only 15% in Project Achilles), the 40 CYCLOPS candidates identified in the Achilles analysis were also among the most significant genes in the shARP analysis (Kolgorov-Smirnov [KS] statistic, p = 2 × 10^-13).

Features of CYCLOPS Genes

In copy number analyses collected from 3,131 tumor samples and cancer cell lines (Beroukhim et al., 2010), each CYCLOPS candidate exhibited hemizygous loss in an average of 18.5% of samples (range, 8%–33%), which was as common as for the other 5,256 genes in the analysis (average, 17.7%; range, 4%–34%; two-tailed permutation test, p = 0.17). In contrast, CYCLOPS genes exhibited much lower rates of homozygous deletion (p = 0.02) and DNA methylation (p = 0.026) (Figure 1E). This observation suggested that CYCLOPS genes are enriched for genes required for cell proliferation or survival.

We also found that CYCLOPS candidates are highly enriched among 1,336 human genes that are homologous to the set of genes found to be essential in S. cerevisiae (Zhang and Lin, 2009) (p < 0.0001) and that exhibit comparable rates of genetic and epigenetic alterations (Table S3). A pathway enrichment analysis showed that the spliceosome, proteasome, and ribosome were the most highly enriched pathways among CYCLOPS candidates (KS statistic FDR = 1.4 × 10^-8, 2.7 × 10^-5, and 1.8 × 10^-4, respectively) and in our analysis of the validation set (FDR = 3.1 × 10^-15, 1.5 × 10^-12, and 2.3 × 10^-17, respectively). Together, these observations indicate that CYCLOPS genes are a unique subset of cell-essential genes
Figure 1. Identification of CYCLOPS Genes

(A) The percentage of the cancer genome involved in copy number loss.

(B) The fraction of deleted regions associated with deletion events of varying lengths.

(C) Biallelic inactivation of a tumor suppressor is often associated with a focal alteration of one copy (red bar) and hemizygous loss of all genes on the chromosome arm containing the other copy.

(D) Schematic describing the approach to identifying CYCLOPS genes. For each gene, we separated cell lines with and without loss of the gene and compared their dependency on that gene by permuting class labels.

(E) Frequency of hemizygous deletion, homozygous deletion, or DNA methylation of CYCLOPS and other genes. Data are presented as averages ± SEM. See also Figure S1 and Tables S1, S2, and S3.
Table 1. Top-Ranked CYCLOPS Candidates and Frequency of Partial Genomic Loss in 3,131 Tumors

<table>
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<tr>
<th>Gene</th>
<th>Band</th>
<th>FDR Value</th>
<th>Frequency of Loss</th>
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<td>PSMC2a</td>
<td>q22.1</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>EIF2B2</td>
<td>14q24.3</td>
<td>0.03</td>
<td>0.17</td>
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<td>EEF2</td>
<td>19p13.3</td>
<td>0.03</td>
<td>0.27</td>
</tr>
<tr>
<td>PHF5b</td>
<td>22q13.2</td>
<td>0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>HPGD</td>
<td>4q34.1</td>
<td>0.03</td>
<td>0.26</td>
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<td>RPS15c</td>
<td>1q13.3</td>
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<td>0.28</td>
</tr>
<tr>
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<td>20p13</td>
<td>0.03</td>
<td>0.13</td>
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<tr>
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<tr>
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<td>EEF1A1</td>
<td>6q13</td>
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aProteasome KEGG Pathway designation.
bSpliceosome KEGG Pathway designation.
cRibosome KEGG Pathway designation.

For which partial, but not complete, suppression is compatible with cancer cell viability.

These observations led us to hypothesize that copy number loss might unveil vulnerabilities in CYCLOPS genes through decreased gene expression. We therefore evaluated the relation between copy number loss and expression by using integrated SNP and expression data for 16,767 and 11,118 genes, respectively, in two panels of samples: the Cancer Cell Line Encyclopedia (CCLE of 806 cell lines across 24 cancer types) (Barretina et al., 2012) and 429 ovarian cancers profiled by The Cancer Genome Atlas (TCGA) Project (Cancer Genome Atlas Research Network, 2011). We found that the average strength of correlation between copy number loss and messenger RNA (mRNA) expression was significantly higher for CYCLOPS candidates than for the other genes in our analysis (CCLE, r = 0.39 versus 0.26, p < 0.0001; TCGA, r = 0.44 versus 0.34, p = 0.0017; Figure S1C).

**PSMC2 Is a CYCLOPS Gene**

PSMC2 (Rpt1) was the highest-ranked CYCLOPS candidate in our original analysis and was also significant in the validation data set. PSMC2 is part of the 19S regulatory complex of the 26S proteasome, which is responsible for catalyzing the unfolding and translocation of substrates into the 20S proteasome (Smith et al., 2011). Either one or two 19S regulatory complexes combine with a single 20S catalytic complex to form, respectively, a singly or doubly capped (26S1 or 26S2) complete 26S proteasome (Finley, 2009). PSMC2 expression is essential for 19S and 26S proteasome assembly (Kaneko et al., 2009).

To minimize the possibility that other genetic alteration(s) confounded our analyses, we determined whether expression or copy number levels of every other gene for which we had data showed significant correlations with PSMC2 sensitivity. Low PSMC2 expression (FDR < 0.017) and PSMC2 copy number loss (FDR < 0.008) were the features most significantly correlated with PSMC2 sensitivity (Table S4). Conversely, among the 7,250 genes in our Achilles analysis, sensitivity to PSMC2 was the only feature that correlated with PSMC2 copy number loss (FDR < 0.25; Table S5). In particular, among all 47 other proteasome components surveyed, neither expression levels nor copy number status significantly correlated with PSMC2 sensitivity.

We also found no evidence that suppression of any of the other proteasome components inhibited the proliferation of cell lines with PSMC2 copy number loss. The association between PSMC2 copy number loss and PSMC2 sensitivity also remained significant when cells with PSMC2 copy number gains were excluded from the analysis (p = 0.0006).

To estimate the differential sensitivity of cell lines harboring normal copies or copy number loss of PSMC2 to gene suppression, we compared the effects of PSMC2 suppression to that observed when we suppressed the oncogenes KRAS, PIK3CA, and BRAF. In consonance with prior studies (Weinstein and Joe, 2006), suppression of these oncogenes inhibited proliferation of cells harboring mutated and constitutively active oncogenes compared to cells expressing wild-type oncogenes (p < 2 × 10−5 in each case) (Figure 2A). However, the difference in PSMC2 dependency scores between cell lines with and without PSMC2 copy number loss (PSMC2Loss and PSMC2Neutral, respectively) was greater than for any of these oncogenes (Figure 2A).

We confirmed the vulnerability of PSMC2Neutral lines to PSMC2 suppression in a direct competition assay by comparing the proliferation rate of uninfected cells to cells that coexpress green fluorescent protein (GFP) and either shLacZ or a PSMC2-specific shRNA (Figure S2A) in six ovarian cell lines over 21 days. The expression of shLacZ or PSMC2 shRNAs did not induce significant changes in the proliferation of PSMC2Neutral cells, including two ovarian cancers and one nontransformed immortalized ovarian surface epithelial (IOSE) cell line (Liu et al., 2004) (Figure 2B). After 21 days of culture, PSMC2 levels remained suppressed in PSMC2Neutral cells that constitutively express PSMC2 shRNA, which is consistent with the lack of an observed proliferation deficit (Figure S2B). In contrast, expression of PSMC2 shRNAs in PSMC2Loss cells was not compatible with long-term culture and reduced the proliferation rate by at least 50% in all three PSMC2Loss ovarian cancer cell lines within 7 days (Figure 2B).

To confirm that these observations were due to the suppression of PSMC2, we expressed an N-terminal V5-epitope-tagged form of PSMC2 (hereafter referred to as V5-PSMC2) in OVCA8,
a PSMC2 Loss cell line. V5-PSMC2 expression was unaffected by an shRNA that targets the 3’ untranslated region (UTR) of endogenous PSMC2 and rescued the proliferation of OVCAR8 cells that express this shRNA (Figure 2C). These observations confirmed that partial loss of PSMC2 renders cancer cell lines highly dependent on the remaining PSMC2.

**PSMC2 Levels and Survival in PSMC2 Loss Cell Lines**

The increased vulnerability of PSMC2 Loss lines correlated with both PSMC2 copy number loss and low mRNA expression (Table S4). Expression and copy number of PSMC2 also correlate with each other in both the CCLE ($r = 0.64$) and TCGA ovarian ($r = 0.49$) sample sets (Figure S3A), indicating that cancer cells that have PSMC2 copy number loss tolerate reduced PSMC2 expression.

To explore the effects of PSMC2 loss on PSMC2 protein levels, we evaluated PSMC2 levels in IOSE cells and in ten ovarian cancer cell lines, including five PSMC2 Neutral and five PSMC2 Loss lines. To minimize potential confounding of other genetic events affecting the 19S complex, we selected PSMC2 Neutral lines that had no copy number gains of PSMC2 and PSMC2 Loss lines that had copy number loss of no more than one other 19S regulatory complex subunit (Table S6). All five PSMC2 Loss cell lines expressed lower levels of PSMC2 than any of the other cell lines (Figure 3A). In contrast, the levels of 19S subunits, including PSMC1 (Rpt2), PSMC4 (Rpt3), PSMC6 (Rpt4), PSMC3 (Rpt5), PSMC5 (Rpt6), PSMD2 (Rpn1), PSMD1 (Rpn2), and PSMD4 (Rpn10) or the 20S subunits PSMB5 (β5) and PSMA1–6 (α subunits) failed to correlate with PSMC2 copy number (Figure S3B). Because PSMC2 is essential for cell proliferation, we concluded that PSMC2 Neutral cells either require more PSMC2 or produce more than is necessary for survival. Therefore, we engineered an experimental system to manipulate the levels of PSMC2 expression in both cell types.

Specifically, we expressed a PSMC2-specific shRNA under the control of a doxycycline-regulated promoter in PSMC2 Loss (Dox-shRNA-2 OVCAR8) and PSMC2 Neutral (Dox-shRNA-2 A2780) cells. The addition of doxycycline led to PSMC2 suppression in both cell lines (Figure 3B). Under these conditions, A2780 cells continued to proliferate, whereas OVCAR8 cells arrested in the G2 phase of the cell cycle and died by apoptosis (Figure S3C). To verify that A2780 cells tolerate increased PSMC2 suppression, we varied the degree of suppression by modulating the doxycycline concentration. A 50% decrease in PSMC2 mRNA reduced the proliferation of OVCAR8 cells, but not A2780 cells (Figure 3D), indicating that the PSMC2 Neutral line A2780 expresses more PSMC2 than is required for proliferation.

To determine the amount of PSMC2 required to maintain A2780 cell proliferation, we further suppressed PSMC2 expression by transfecting a pool of three PSMC2-specific siRNAs at varying concentrations. The proliferation of A2780 cells decreased only when PSMC2 expression was suppressed by more than 60% (Figures S3D and S3E). By using quantitative RT-PCR and immunoblotting, we estimated that untreated

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**Figure 2. PSMC2 Loss Cells Are Sensitive to PSMC2 Suppression**

(A) Comparison of gene dependence between three models of oncogene addiction and PSMC2. Cell lines were classified by mutation status for PIK3CA, BRAF, or KRAS (n = 102 in each case) or PSMC2 copy number (n = 84). For each class, gene dependency scores reflect the sensitivity to the gene on which the categorization was based. Solid bars represent average scores.

(B) The effect of PSMC2 suppression on the proliferation of six ovarian cell lines. Exogenous gene

(C) PSMC2 levels (left) and relative proliferation rates (right) among cells expressing different combinations of PSMC2 shRNA targeting the 3’ UTR and ectopic V5-PSMC2 expression.
OVCAR8 cells express ~50% of the PSMC2 mRNA and protein found in A2780 cells (Figures S3F and S3G) and that both A2780 and OVCAR8 lose proliferative capacity at similar total levels of PSMC2 expression (Figure 3E), suggesting that they have a comparable threshold requirement for PSMC2.

To determine whether partial loss of PSMC2 affects the sensitivity of cells to suppression of other members of the 19S complex, we used an isogenic system in which Dox-shLacZ and Dox-shRNA-2 cells were cultured in doxycycline (30 ng/ml) so that shRNA-2 cells express levels of PSMC2 comparable to

Figure 3. Threshold Requirement for PSMC2
(A) PSMC2 levels among ovarian cancer cell lines.
(B) PSMC2 levels in cells that express an inducible shRNA that targets either PSMC2 or LacZ.
(C) Effects of PSMC2 suppression on proliferation.
(D) Relationship between PSMC2 mRNA expression and proliferation in PSMC2Neutral (left) and PSMC2Loss (right) cells. Data represent averages ± SD.
(E) Schematic combining data from Figures 3D, S3D, and S3E indicate that A2780 and OVCAR8 cells share a similar absolute threshold requirement for PSMC2 (dashed line).
(F) Cellular proliferation in A2780 cells with and without PSMC2 suppression after introduction of control, PSMC2, or PSMC5 siRNAs.

Data are presented as averages ± SEM. See also Figure S3 and Table S6.
PSMC2\textsuperscript{loss} cells. Under these conditions, both Dox-shLacZ and Dox-shRNA-2 proliferated at comparable rates. We then suppressed the expression of either PSMC2 or PSMC5 by introducing siRNA targeting these genes at concentrations that did not affect the expression of other 19S components (Figure S3H). As expected, further suppression of PSMC2 in Dox-shRNA-2 cells inhibited proliferation as compared to Dox-shLacZ cells (Figure 3F). In contrast, suppression of PSMC5 led to a comparable inhibition of cell proliferation in both Dox-shLacZ and Dox-shRNA-2 cells. Suppression of PSMC2 also did not affect the expression of other 19S components (Figure S3I). Together, these observations indicate that partial loss of PSMC2 sensitizes cells to further suppression of PSMC2, but not of other 19S proteasome components.

**PSMC2\textsuperscript{loss} Cells Exhibit Only Slight Alterations in Proteasome Content and Function**

The tolerance of cells for loss of PSMC2 copy number and expression indicates that cells contain a reservoir of excess PSMC2 that is not required for proliferation. This reservoir may be maintained in an excess of fully assembled 26S proteasome or elsewhere in the cell. We analyzed proteasome assembly and content by performing PAGE on crude lysates under native (nondenaturing) conditions. Under these conditions, the 26S proteasome complex is stable and active and migrates in two distinct bands, which are distinguished by having either one or two 19S subunits incorporated in the formation of the 26S (Elsasser et al., 2005). By using lysates collected from IOSE, two PSMC2\textsuperscript{neutral}, and three PSMC2\textsuperscript{loss} cancer cell lines (all with comparable proliferation rates), we detected 26S\textsubscript{1}, 26S\textsubscript{2}, and 20S proteasome complexes by immunoblotting for the core 20S subunits, PSMA1–6 (Figure 4A). By using lysates collected from IOSE, two PSMC2\textsuperscript{neutral}, and three PSMC2\textsuperscript{loss} cancer cell lines (all with comparable proliferation rates), we detected 26S\textsubscript{1}, 26S\textsubscript{2}, and 20S proteasome complexes by immunoblotting for the core 20S subunits, PSMA1–6 (Figure 4A).

We found that PSMC2\textsuperscript{loss} lines express only slightly less 26S proteasome (most evident in 26S\textsubscript{2}), which is not comparable to the decrease in PSMC2 in these cells (Figure 4A), and increased 20S proteasome. Similarly, comparable changes in PSMC2 expression in isogenic systems failed to substantially affect 26S

**Figure 4. PSMC2\textsuperscript{loss} Cells Lack a PSMC2 Reservoir**

(A) Total PSMC2 levels (top) and native PAGE immunoblot for PSMA1–6 (middle) in PSMC2\textsuperscript{neutral} and PSMC2\textsuperscript{loss} cells.

(B) Native PAGE immunoblot for PSMA1–6 in A2780 (left) and OVCAR8 (right) after inducible suppression or ectopic expression of PSMC2, respectively.

(C) Native PAGE 26S and 20S peptidase cleavage in PSMC2\textsuperscript{neutral} and PSMC2\textsuperscript{loss} cells.

(D) Native PAGE 26S and 20S peptidase cleavage in isogenic systems used in (B).

(E) In vitro 26S proteasome activities in PSMC2\textsuperscript{neutral} and PSMC2\textsuperscript{loss} cells. Each point represents a cell line; dashed lines represent averages.

(F) In vitro 26S proteasome activities in isogenic systems used in (B) and (D).

(G and H) Dose response curve for bortezomib in (G) A2780 cells with and without PSMC2 suppression and (H) OVCAR8 with and without ectopic V5-PSMC2 expression.

See also Figure S4 and Table S7.
proteasome content. Suppression of PSMC2 levels by 50% in the Dox-shRNA-2 A2780 system led to an increase in the 20S complex but little to no change in 26S1 (Figure 4B) or 26S2 (Figure S4A) proteasome content relative to controls. Conversely, ectopic expression of PSMC2 in OVCAR8 cells led to a slight reduction in 20S levels and slight increases in 26S1 and 26S2 proteasome content (Figures 4B and S4A). The levels of other 19S proteasome units remained unchanged (Figure S4B).

Similarly, peptidase cleavage activity varied only slightly between PSMC2Neutral and PSMC2Loss lines. We observed the greatest differences in in-gel analyses of peptidase activity, which revealed less 26S2 proteasome peptidase cleavage and increased 20S peptidase activity in PSMC2Loss cells (Figure 4C). These changes were recapitulated by PSMC2 suppression in A2780 cells and were reversed by ectopic PSMC2 expression in OVCAR8 cells (Figure 4D). The decrease in 26S2 activity in PSMC2Loss relative to PSMC2Neutral cells, however, was not associated with significant differences in peptidase cleavage when quantitatively assayed in whole-cell lysates under conditions (in the absence of SDS) in which free 20S proteasome does not contribute activity (Kisselev and Goldberg, 2005) (p = 0.39) (Figure 4E). In this assay, proteasome-specific peptidase activity is determined by bortezomib-inhibited cleavage. We found that 97% of activity was ablated by bortezomib, suggesting that other proteases did not contribute substantially to the measured activity. Lysates from PSMC2Neutral and PSMC2Loss lines grown under conventional nonstressed conditions also exhibited qualitatively similar total levels of polyubiquitin (Figure S4D).

To test the acute effect of manipulating PSMC2 expression on peptidase activity, we measured peptidase activity in lysates of A2780 cells in which we suppressed PSMC2 and lysates of OVCAR8 cells engineered to recover PSMC2 expression. Suppression of PSMC2 by 50% in A2780 cells led to a 17% reduction in total 26S specific peptidase activity, which is associated with reduced 26S2 activity (Figure 4F). Conversely, ectopic PSMC2 expression in OVCAR8 led to a 15% increase in peptidase activity, which is associated with increased 26S2 activity. The finding in both systems—that modulating PSMC2 levels by up to 50% resulted in only a 17% alteration in 26S activity—suggested that PSMC2 content was not the limiting component to 26S formation in PSMC2Neutral cells.

Across 133 cell lines previously tested, we found no increased sensitivity to bortezomib in PSMC2Loss cells and found no significant correlation between the concentration of bortezomib that inhibits proliferation by 50% (IC50) and decreased expression of any of the 47 26S proteasome components (Garnett et al., 2012) (Table S7). Suppression of PSMC2 in Dox-shRNA-2 A2780 cells or ectopic PSMC2 expression in OVCAR8 cells also did not substantially affect the bortezomib IC50 (Figures 4G and 4H). These observations are consistent with our prior observation that 26S proteasome function is not substantially compromised in PSMC2Loss cells.

**PSMC2Neutral Cells Have a Reservoir of PSMC2 that Buffers 26S Proteasome Levels against PSMC2 Loss**

The finding that PSMC2Neutral cells have near-equal 26S proteasome content to PSMC2Loss cells, even though they express higher levels of PSMC2, suggests that PSMC2Neutral cells contain a separate reservoir of PSMC2 that is preferentially lost when levels are reduced. To identify this reservoir, we combined native PAGE with immunoblotting for PSMC2 across a panel of cell lines (Figure 5A). Of the multiple reactive bands identified, even after a long exposure, only one band (ComplexPSMC2) was present in all of the PSMC2Neutral lines, but none of the PSMC2Loss lines. By using isogenic systems, we also found that PSMC2 suppression in Dox-shRNA-2 A2780 cells led to reduced levels of ComplexPSMC2, whereas ectopic PSMC2 expression in OVCAR8 cells led to its reappearance (Figure 5B). These results suggest that ComplexPSMC2 is a specific PSMC2 reservoir.

We hypothesized that ComplexPSMC2 serves as a “buffer” in PSMC2Neutral cells, enabling such cells to maintain 26S proteasome levels and function in the face of reduced PSMC2 expression. In this case, PSMC2 suppression should deplete ComplexPSMC2 before reducing 26S proteasome levels. To quantify the consequences of reducing PSMC2 on ComplexPSMC2 and 26S proteasome levels, we compared dilutions of lysates from Dox shRNA-2 A2780 cells propagated in the absence of doxycycline to lysate collected from these cells cultured in doxycycline (Figure 5C). In cells in which PSMC2 was suppressed, the relative loss of ComplexPSMC2 exceeded the decrease in 26S proteasome content. These observations indicate that ComplexPSMC2 was preferentially lost in A2780 cells after PSMC2 suppression. In contrast, PSMC2 suppression in OVCAR8 cells, which lack ComplexPSMC2, led to near-complete ablation of 26S proteasome levels and peptidase activity and led to a qualitative increase in the amount of polyubiquitin (Figures 5D–5F, S5A, and S5B).

To analyze the components of ComplexPSMC2, we fractionated lysates from ISE cells expressing either V5-GFP or V5-PSMC2 (Figure S5C) by using a glycerol gradient (Figure S5D) and isolated V5-immune complexes containing either ComplexPSMC2 or 26S proteasome. ComplexPSMC2 immune complexes (collected in fractions 2–4) contained PSMC2, PSMC1 (Rpt2), PSMD2 (Rpn1), and PSMD5 (SS5) (Figure 5G), which are subunits of one of three complexes known to compose the base of the 19S proteasome (Funakoshi et al., 2009; Kaneko et al., 2009; Park et al., 2009; Roelofs et al., 2009; Saeki et al., 2009; Thompson et al., 2009). ComplexPSMC2 did not contain subunits of the other two complexes, PSMC3 (Rpt5), PSMC4 (Rpt3), PSMC5 (Rpt6), and PSMC6 (Rpt4), or members of the 20S proteasome, PSMB5 (I5) or PSMA1–6 (α subunits) (Figure 6C). All of these proteins, except PSMD5, were detected in immune complexes containing the 26S complex (from fractions 7–9). These observations indicate that the PSMC2 reservoir is a subcomplex of the 26S proteasome.

**The Reduction of PSMC2 Levels in PSMC2Loss Cells Inhibits Orthotopic Tumor Growth**

To explore the therapeutic potential of PSMC2 suppression in vivo, we tested the consequences of suppressing PSMC2 in ovarian xenografts. Specifically, we used a tumor-targeted nanoparticle delivery system that delivers small interfering RNA (siRNA) into the cytosol of cells within the tumor parenchyma (Ren et al., 2012). We generated tumor-penetrating
nanocomplexes (TPNs) consisting of PSMC2-specific siRNA noncovalently bound to tandem peptides bearing an N-terminal cell-penetrating domain, Transportan (TP), and a C-terminal tumor-specific domain, LyP-1 (CGNKRTRGC), which binds to its cognate receptor p32 (Figure 6A).

We first assessed the compatibility of cell lines with TPN-targeted siRNA delivery. OVCAR8 and A2780 cells exhibited high cell surface levels of expression of p32, whereas IOSE cells exhibited low expression (Figure S6A). In consonance with these observations, flow cytometry to quantify cytosolic delivery of fluorescently labeled siRNAs indicated substantial accumulation of siRNA in both OVCAR8 and A2780 cells (Figure 6B). A monoclonal antibody directed against p32 (monoclonal antibody [mAb] 60.11) substantially reduced nanocomplex uptake, whereas a control antibody had no effect on uptake. These results indicate that surface p32 expression correlates with enhanced uptake of TPNs and that TPN-mediated siRNA delivery is p32 receptor specific.

We next used these TPNs to confirm the vulnerability of PSMC2Loss cells to PSMC2 suppression both in vitro and in vivo. We treated OVCAR8 and A2780 cells in vitro with TPNs carrying siRNAs targeting nonoverlapping exons of PSMC2. In both cell types, we observed a reduction of PSMC2 protein relative to cells treated with TPNs carrying GFP siRNA (Figure S6B). This reduction was associated with a corresponding decrease in proliferation only in the OVCAR8 cells (Figure S6C). We then used these TPNs to treat mice harboring orthotopic OVCAR8 or A2780 tumors expressing firefly luciferase. We injected TPNs carrying PSMC2-siRNA (1 mg siRNA/kg body weight for 14 days) intraperitoneally...
Figure 6. Tumor-Penetrating Nanocomplex-Mediated Delivery of PSMC2-Specific siRNA Suppresses Ovarian Tumor Growth

(A) Schematic depicting the mechanism of TPN-mediated delivery of siRNA.

(B) Comparison of cellular uptake of fluorescently labeled siRNA in untreated cells (solid gray) and cells treated with TPN alone (black line) and in combination with IgG (gray line) or an antibody to p32 (solid pink).

(C) Tumor burden of mice bearing disseminated OVCAR8 (top) or A2780 (bottom) orthotopic xenografts treated with TPN carrying either GFP-siRNA or PSMC2-siRNA. n = 5 animals per group.

(D) PSMC2 levels in orthotopic tumors of A2780 or OVCAR8 after treatment with nanoparticles carrying siGFP or siPSMC2.

(E) Tumor burden of mice bearing orthotopic tumors of OVCAR8 cells expressing V5-PSMC2. n = 5 animals per group.

(F) Tumor burden (top) and overall survival (bottom) of mice bearing orthotopic tumors of A2780 cells expressing doxycycline-inducible shRNA against PSMC2. n = 5–13 animals per group.

Data in all panels are presented as average ±SEM. Significance was determined by one-way analysis of variance (ANOVA) or log rank (Mantel-Cox) tests as appropriate. n.s., not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. See also Figure S6.
are limited by 19S regulatory complex levels (Figure 4A). The complex, but not 19S, suggesting that 26S proteasome levels and normal cells. For example, cells often express free 20S

By integrating data derived from the genomic characterization of human tumors with systematic interrogation of essential genes in particular and of CYCLOPS genes in general renders cells highly dependent on the nucleotide excision DNA repair pathway are highly dependent on the nucleotide excision DNA repair pathway (Bryant et al., 2005; Farmer et al., 2005)—provide evidence that synthetic lethality may be clinically useful. Targeting CYCLOPS genes represents a different approach to synthetic lethality. In this case, the intervention is lethal to cells with a genetic event that is independent of that event’s effect on the pathways that drive cancer.

Advances in cancer therapeutics benefit from our ability to identify vulnerabilities predicted by genomic features that are unique to cancer cells. Indeed, the inhibition of recurrent activating mutations in proto-oncogenes has led to several new cancer treatments. The cancer-specific vulnerabilities we have identified herein are the consequence of alterations in genes affected by genomic disruption that may have no consequence to the process by which the cell transformed or continues to proliferate. These genomic alterations are more frequent than most known driver alterations, occur across lineages, and could theoretically be targeted in a large number of patients.

Although individual CYCLOPS candidates such as PSMC2 will require further investigation in human subjects, the 56 candidate genes we identified may be an underestimate of the true number of potential targets. Our initial Project Achilles analysis included only 5,312 genes, and many of these genes may represent false negative results due to insufficiently effective shRNAs. The set of 86 cell lines was not large enough to enable detection of lineage-specific CYCLOPS genes. Indeed, we identified additional...
CYCLOPS targets in an independently generated RNAi data set enriched in breast and pancreatic lineages, in addition to validating the targets described in our more lineage-diverse data set. Systematic evaluation of the completely annotated genome using more shRNAs for each gene and a larger group of cell lines representing many lineages is likely to uncover many more potential targets.

Besides copy number loss, other types of genomic alteration may also unveil CYCLOPS vulnerabilities. In most cases, vulnerability to suppression of CYCLOPS genes was associated with decreased expression. Other events may also decrease expression of essential genes, including sequence variants, epigenetic modification, or chromosome translocations. Any of these mechanisms may lead to cancer-specific vulnerabilities. Further work will be necessary to explore these other classes and to define the role of CYCLOPS targets in cancer therapy.

EXPERIMENTAL PROCEDURES

Copy Number and Methylation Analysis of Tumors
Copy numbers were determined for 3,131 cancer samples as previously described (Mermel et al., 2011). Marker and gene locations were based on the hg18 genome build. The criteria used to define partial copy number loss, homozygous deletion, and the length of each deletion are detailed in the Extended Experimental Procedures. Gene-level DNA methylation \( \beta \) values were collected for 601 ovarian tumors from the TCGA web portal. Genes with \( \beta \) values >0.7 were considered methylated. Genes missing data in any sample were excluded from the analysis.

CYCLOPS Analysis
For each cell line, we classified each gene as intact (no copy number loss) or partial loss or to be excluded (for genes undergoing homozygous loss or with ambiguous data) based on thresholds determined by using the distribution of relative copy numbers generated from analysis of SNP array data for that cell line (see Extended Experimental Procedures). Gene dependency scores were determined by using the ATARiS algorithm (see Extended Experimental Procedures). The statistical significance of mean gene dependency score differences between intact and partial loss cell lines was determined by comparing the observed data to data representing 50,000 random permutations of class labels, each maintaining the number of cell lines and lineage distribution in each class. Multiple hypotheses were corrected by using the FDR framework (Benjamini and Hochberg, 1995).

26S Proteasome Activity
We measured excitation-emission spectra (360 nm to 430 nm) during incubation in vitro at 37°C every 30 s for 1 hr for a 100 μl solution containing 5 μl of lysate (buffer A) in 50 mM Tris-HCl (pH 8.0), 40 mM KCl, 5 mM MgCl2, 1 mM ATP, 1 mM dithiothreitol (DTT), and 100 μM Sucrose-LLVY-AMC (Bachem). We converted these measurements to amount of peptide cleavage by using a standard curve generated from the excitation-emission spectra of AMC (Bachem). Samples were tested in triplicate with and without the addition of 1 μM bortezomib. The average value of peptide cleavage in the bortezomib sample was subtracted to determine 26S proteasome activity.

Native Gel Analysis for Proteasome Content or Proteasome Activity
10 μl of lysate (buffer A) was loaded onto 3%–8% Tris-Acetate PAGE (Invitrogen) and run in Tris-Glycine at 4°C and 60V for 17 hr. Gels were transferred to nitrocellulose membranes in Tris-Glycine at 70V for 4 hr for immunoblotting or in-gel peptidase activity. The latter was performed by incubating with gentle agitation in 50 mM Tris-HCl (pH 8.0), 5 mM MgCl2, 1 mM ATP, 1 mM DTT, and 50 μM Suc-LLVY-AMC (Bachem) at 37°C for 30 min. Gels were visualized under UV transillumination. Following photography of 26S proteasome activity, gels were incubated for another 45 min at 37°C in the same buffer with the addition of 0.2% SDS and reanalyzed by UV transillumination to assess 20S peptidase activity.

Generation of PSMC2-Specific and Control siRNA Nanoparticles
We measured excitation-emission spectra (360 nm to 430 nm) during incubation in vitro at 37°C every 30 s for 1 hr for a 100 μl solution containing 5 μl of lysate (buffer A) in 50 mM Tris-HCl (pH 8.0), 40 mM KCl, 5 mM MgCl2, 1 mM ATP, 1 mM dithiothreitol (DTT), and 100 μM Sucrose-LLVY-AMC (Bachem). We converted these measurements to amount of peptide cleavage by using a standard curve generated from the excitation-emission spectra of AMC (Bachem). Samples were tested in triplicate with and without the addition of 1 μM bortezomib. The average value of peptide cleavage in the bortezomib sample was subtracted to determine 26S proteasome activity.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Extended Experimental Procedures, six figures, and seven tables and can be found with this article online at http://dx.doi.org/10.1016/j.cell.2012.07.023.

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