Genomic Complexity Profiling Reveals That HORMAD1 Overexpression Contributes to Homologous Recombination Deficiency in Triple-Negative Breast Cancers

ABSTRACT

Triple-negative breast cancers (TNBC) are characterized by a wide spectrum of genomic alterations, some of which might be caused by defects in DNA repair processes such as homologous recombination (HR). Despite this understanding, associating particular patterns of genomic instability with response to therapy has been challenging. Here, we show that allelic-imbalanced copy-number aberrations (AiCNA) are more prevalent in TNBCs that respond to platinum-based chemotherapy, thus providing a candidate predictive biomarker for this disease. Furthermore, we show that a high level of AiCNA is linked with elevated expression of a meiosis-associated gene, HORMAD1. Elevated HORMAD1 expression suppresses RAD51-dependent HR and drives the use of alternative forms of DNA repair, the generation of AiCNAs, as well as sensitizing cancer cells to HR-targeting therapies. Our data therefore provide a mechanistic association between HORMAD1 expression, a specific pattern of genomic instability, and an association with response to platinum-based chemotherapy in TNBC.

SIGNIFICANCE: Previous studies have shown correlation between mutational “scars” and sensitivity to platinums extending beyond associations with BRCA1/2 mutation, but do not elucidate the mechanism. Here, a novel allele-specific copy-number characterization of genome instability identifies and functionally validates the inappropiate expression of the meiotic gene HORMAD1 as a driver of HR deficiency in TNBC, acting to induce allelic imbalance and moderate platinum and PARP inhibitor sensitivity with implications for the use of such “scars” and expression of meiotic genes as predictive biomarkers.

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INTRODUCTION

Women with germline mutations in either BRCA1 or BRCA2, the products of which are required for DNA double-strand break repair by homologous recombination (HR; refs. 1, 2), have an increased risk of developing breast and ovarian cancers. BRCA1 mutations are particularly prevalent in triple-negative breast cancers (TNBC), a subtype of breast cancer defined by a lack of elevated HER2, estrogen receptor (ER), and progesterone receptor expression, and molecularly similar high-grade serous ovarian cancers (HGSC; ref. 3). Many TNBCs and HGSCs are characterized by high mitotic indices and highly unstable genomes, observations that have stimulated research into chromosomal instability as a biomarker of response to platinum-based chemotherapies, and also into synthetic lethal agents such as the poly (ADP-ribose)-polymerase (PARP) inhibitors (4, 5). The potential causes and consequences of chromosomal instability phenotypes are likely to be diverse and encompass structural-level copy-number aberrations (CNA) and loss of heterozygosity (LOH; ref. 6). High-resolution single-nucleotide polymorphism (SNP) arrays and associated methods of analysis are now commonly used to detect CNAs and LOH in tumors (7, 8). In the context of TNBC, several array-based CNA/LOH signatures of platinum-based drug response and BRCA1/2 inactivation have recently been developed (9–12). These include a telomeric allelic imbalance score (NtAI) that predicts sensitivity to platinum analogues (10); a homologous repair defect (HRD) score designed to comprehensively assess the impairment of HR in addition to BRCA1 and BRCA2 deficiency (9); the large-scale transition measurement, a signature of BRCA1 inactivation–associated genomic instability (11); as well as an array-comparative genomic hybridization-based classifier designed to identify germline BRCA1/2-mutant carriers (13). Although promising, current measures do not suggest mechanisms that either compensate for underlying repair deficiencies or drive the observed genomic instability. Moreover, the observation that BRCA1/2 inactivation is not the only driver of genomic instability in TNBC (14, 15) has prompted the search for alternative mechanisms that drive HR dysfunction and subsequent chromosomal instability.

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Here, we demonstrate that scores of allelic imbalance are higher in TNBCs responding to platinum-based chemotherapy. Furthermore, we identify and functionally validate HORMAD1, a cancer testis antigen involved in the promotion of nonconservative recombination in meiosis (16–18), as a novel driver of the allelic imbalance phenotype in TNBC. HORMAD1 mediates these effects through suppression of RAD51-dependent HR, and in doing so drives 53BP1-dependent nonhomologous end joining (NHEJ). In addition, HORMAD1 expression correlated with a better response to HR defect-targeting agents in both TNBC cell lines and clinical trial data, and may add value to BRCA1/2 mutation testing for platinum treatment in unselected patients with TNBC.

RESULTS

TNBCs Exhibit Distinct Types and Levels of Chromosome Scarring

To comprehensively capture the variety of genomic aberrations that TNBCs exhibit, we interrogated the genome-wide Affymetrix SNP6.0 array profiles of an unpublished cohort of 126 TNBCs from Guy’s Hospital King’s College London (KCL TNBCs; London, United Kingdom). We obtained allele-specific copy-number profiles for 111 of these samples, and confirmed several previously identified recurrent (>25% of cases) gains, losses, and regions of copy-number–neutral LOH (CnLOH; Supplementary Fig. S1A–S1C; refs. 12, 19). Furthermore, approximately 13% of KCL TNBCs (Supplementary Table S1) harbored chromothripsis-like features (20). In order to unravel this complexity and extract genomic patterns to provide both biologic and clinical insights, we devised three categories of genomic scars that might each be generated by a distinct combination of mutational processes: (i) allelic-imbalanced CNAs (S_{AiCNA}), which potentially reflect defects arising from an increased reliance on error-prone forms of double-strand break repair such as NHEJ or nonallelic HR; (ii) copy-number–neutral LOH (S_{CnLOH}), which might arise from regions of nonconservative allelic HR (6, 21, 22); and (iii) allelic-balanced CNAs (S_{AbCNA}), which may be indicative in large part of whole-genome doubling (Methods; Supplementary Fig. S2; Supplementary Table S2; ref. 23). In addition, we derived a combined score, S_{Ai} (the sum of S_{AiCNA} and S_{CnLOH}), to capture impaired conservative double-strand break repair irrespective of the precise biologic mechanism or mechanisms that created it (10). All four scores were collectively termed “scores of chromosomal instability scarring” (SCINS).

We applied SCINS to our KCL TNBCs and observed that the total burden as well as the relative degree of S_{AiCNA}, S_{CnLOH}, and S_{AbCNA} differed substantially over the cohort. Although some TNBCs showed no SCINS-defined genomic alterations, many harbored numerous scars, with one possessing a combined allelic imbalance score (S_{Ai}) of approximately 80 (Fig. 1A). For two TNBCs, the scarring burden was entirely attributable to S_{AiCNA}, whereas others showed an almost 99% contribution from S_{CnLOH}, and some exhibited a roughly equal share of S_{AiCNA} and S_{CnLOH} (Fig. 1A). These results were not influenced by normal cell contamination, because no association between tumor content and any of the SCINS was observed (P > 0.5, Kruskal–Wallis test). A less chromosomally biased distribution was noted for S_{AiCNA} and S_{CnLOH} (Fig. 1A, heatmap; Supplementary Fig. S3A–S3C), whereas genomic segments contributing to S_{CnLOH} were concentrated on chromosomes 14 and 17 (Fig. 1A, heatmap; Supplementary Figs. S3A–S3C and S4A–S4D).

To investigate whether S_{AiCNA}, S_{CnLOH}, and S_{AbCNA} were comparable across different datasets, we obtained copy-number profiles for 97 METABRIC TNBCs (24), 80 TNBCs from The Cancer Genome Atlas (TCGA; ref. 25), and 71 pretreated TNBCs from the gemcitabine-, carboplatin-, and iniparib-treated neoadjuvant PrECOG 0105 (NCT00813956; ref. 26) study (PrECOG TNBCs), as well as a panel of 38 breast cell lines, 20 of which were triple-negative. We applied SCINS to these datasets and demonstrated that overall, our scores were recapitulated. Pairwise comparisons of the core SCINS measures between the four TNBC cohorts and the panel of cell lines revealed no significant difference between the distributions of S_{CnLOH} and S_{Ai} (Fig. 1B). However, whereas the distribution of S_{AiCNA} was similar among TNBCs, and likewise for S_{AbCNA} (P > 0.15, Kolmogorov–Smirnov test; Fig. 1B), the distributions in ER-positive cell lines differed significantly (P < 0.05, Kolmogorov–Smirnov test; Fig. 1B), reinforcing evidence for the particular similarities in genomic instability profiles between TNBCs and their cell line models (27).

Correlation analyses provided validation that each scar was capable of capturing independent information with only moderate correlation observed at most. We found S_{AiCNA} to be largely independent of S_{CnLOH} across all datasets (Fig. 1C). To assess the relationship between SCINS and published measures of HR deficiency and platinum response, we also scored tumors for the previously described N_{Ai} measure of telomeric allelic imbalance (10) and an approximation of the HRD score (9), which we term S_{LOH}. As expected, our composite
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Spearman's rank correlation coefficient

KCL TNBCs

METABRIC TNBCs

PrECOG TNBCs

PrECOG TNBCs

PrECOG TNBCs

Mutated Wild-type Wild-type Mutated Platinum nonresponders Platinum responders

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allelic imbalance score, $S_{\text{Ai}}$, most closely tracked $N_{\text{Ai}}$, whereas a modest correlation was observed between $S_{\text{GalO}}$, and $S_{\text{Ai}}$, and $S_{\text{CnLOH}}$ (Fig. 1C). These results support the segregation of the constituent scores of SCINS as a means of providing different forms of information on the nature of genomic instability in such tumors.

**Platinum Agent Sensitivity Correlates with $S_{\text{AiCNA}}$ and $S_{\text{Ai}}$ in Tumors**

Platinum salts have emerged as potentially selective therapeutics for the treatment of TNBC (28). Because the repair of platinum adducts can induce single- and double-stranded DNA breaks that require HR for their repair, we sought to test whether our measures of genome instability correlated with any specific sensitivity to carboplatin response (Methods) in the PrECOG TNBCs and a cohort of HGSCs from TCGA where whole-genome SNP profiles for such data were available. Our composite allelic imbalance scar, $S_{\text{Ai}}$, proved to be significantly higher in cancers responding to platinum-based chemotherapy (Fig. 1D). We next evaluated the individual contributions made by the two constituent components of $S_{\text{Ai}}$, $S_{\text{AiCNA}}$ and $S_{\text{GalO}}$. In the PrECOG TNBC study, $S_{\text{AiCNA}}$, but not $S_{\text{GalO}}$, was significantly associated with platinum-based chemotherapeutic response (Fig. 1E and F), whereas both $S_{\text{AiCNA}}$ and $S_{\text{GalO}}$ were significantly linked with an enhanced platinum-based chemotherapeutic response in HGSC (Fig. 1E and F). Stratification by $BRCA1/2$ status revealed higher levels of allelic imbalance in $BRCA1/2$-mutated cancers overall, supporting the concept that $S_{\text{AiCNA}}$ is indicative of HR deficiency. Interestingly, among the wild-type $BRCA1/2$ tumors in both cohorts, those patients who responded to platinum treatment had higher $S_{\text{AiCNA}}$ and $S_{\text{Ai}}$ (Fig. 1D and E). Taken together, our results support the potential utility of allelic imbalance–based measures as markers of response to DNA-damaging chemotherapeutics but further highlight the particular contribution of $S_{\text{AiCNA}}$ in driving much of the association with response in TNBC. Of note, the presence of a substantial number of platinum-responsive, high $S_{\text{Ai}}$ tumors without $BRCA1/2$ mutation underscores the fundamental need to identify alternative mechanisms at play that underpin this form of chromosomal instability and its association with platinum sensitivity.

**SCINS-Based Class Discovery Identifies High HORMAD1 Expression Associated with Allelically Imbalanced Tumors**

Next, we sought to identify candidate drivers of the SCINS-defined clusters by interrogating the transcriptional profiles associated with each. Therefore, we conducted hierarchical clustering of the KCL, METABRIC, and PrECOG TNBCs separately using $S_{\text{AiCNA}}$, $S_{\text{GalO}}$, and $S_{\text{AiCNA}}$ as covariates. Matching gene expression data were available for 77, 97, and 59 KCL, METABRIC, and PrECOG TNBCs, respectively. In all datasets, we observed two stable top-level clusters, which divided the cohort into a major cluster of approximately 60% of samples, and a minor group of TNBCs scoring low for all SCINS, referred to hereafter as “Lo-SCINS” (Fig. 2A and B, green branch and Supplementary Fig. S5A). The major group further separated into two distinct and robust subclusters (Fig. 2A and B and Supplementary Fig. S5A). Examination of the three core SCINS across these two subclusters revealed that in both cohorts, one cluster (Fig. 2A and B, blue branches and Supplementary Fig. S5A) comprised samples with high $S_{\text{GalO}}$ (Fig. 2A and B and Supplementary Fig. S5A, box plots), which we termed “Hi-CnLOH.” In contrast, the second subcluster (Fig. 2A and B, red branches and Supplementary Fig. S5A) was composed of a third of samples and featured tumors with the highest $S_{\text{AiCNA}}$ (Fig. 2A and B and Supplementary Fig. S5A, box plots), hereafter termed “Hi-AiCNA.” In contrast, $S_{\text{AiCNA}}$ did not significantly differ between the Hi-AiCNA and Hi-CnLOH clusters. On the basis of these stable SCINS-defined TNBC clusters, we next sought to define some of the transcriptional events associated with these different genomic instability measures. Starting with the top-level partitions, we performed significance analysis of microarray (SAM) and identified $HORMAD1$, a cancer testis antigen that is normally exclusively expressed in germline cells and known to have function in meiosis, to be the top-ranked differentially expressed gene by fold change among tumors in the Hi-AiCNA/Hi-CnLOH cluster of the KCL cohort (Fig. 2C and Supplementary Table S3). In agreement, higher expression of $HORMAD1$ was also seen in the Hi-AiCNA/Hi-CnLOH cluster of the METABRIC and PrECOG TNBC cohorts (Fig. 2D; Supplementary Fig. S5B; Supplementary Table S3) despite only modest correlations between the gene expression values of the datasets (Supplementary Fig. SSC).

Having established a link between $HORMAD1$ expression and our Hi-AiCNA/Hi-CnLOH group of tumors, we next set out to refine this analysis by investigating which genes were associated with distinct forms of allelic imbalance. We interrogated the transcriptional profiles of the Hi-AiCNA, Hi-CnLOH, and Lo-SCINS clusters (Fig. 2E and F), compared the resultant gene lists from each cohort, and found 19, 5, and 45 genes to be common to the Lo-SCINS, Hi-AiCNA, and Hi-CnLOH–specific clusters, respectively (Fig. 2G and Supplementary Table S3), with $HORMAD1$ present in the common gene list of the Hi-AiCNA clusters (Fig. 2H), suggesting a specific association with $S_{\text{AiCNA}}$ scarring. A second gene involved in meiotic recombination, $PSMC3IP$, was also present among genes associated with the high $S_{\text{AiCNA}}$ clusters.

Investigation of the expression pattern of $HORMAD1$ revealed a clear bimodality among TNBCs (Fig. 3A), allowing dichotomization of the population with approximately 60% showing high $HORMAD1$ expression in each cohort (46 of 77 for KCL, and 61 of 97 for METABRIC). Using gene expression signatures as surrogate markers of different aspects of genomic instability and genetic lesions known to be important in TNBC (Supplementary Table S4), we found many of these signature scores to be significantly higher in the high $HORMAD1$ in comparison with the low $HORMAD1$ group of samples (Fig. 3B and Supplementary Fig. S6A–S6J). Examination of $HORMAD1$ expression across all breast cancer types in the KCL (29), METABRIC (24), and TCGA (25) cohorts provided further evidence for a high $HORMAD1$ subgroup, specifically in basal-like breast carcinomas (Fig. 3C). In contrast, we found no significant link between $HORMAD1$ expression and any of the genomic scarring measures in HGSCs ($P > 0.05$; data not shown) or any evidence of bimodality.

Our finding of significant associations between signatures of genomic instability–linked pathways, such as...
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**Figure 2.** SCINS-defined tumor classes show distinct transcriptomic profiles. A and B, heatmaps (yellow-red image) and dendrograms showing the results of clustering 77 TNBCs from the KCL dataset (A) and 97 TNBCs from the METABRIC dataset (B), according to $S_{\text{AbCNA}}$, $S_{\text{CnLOH}}$, and $S_{\text{HiCnLOH}}$. The three TNBC clusters identified for each dataset include Lo-SCINS (green arm of dendrogram), Hi-AiCNA (red arm of dendrogram), and Hi-CnLOH (blue arm of dendrogram). Scores were standardized by row mean, and a color scale is shown to the left of each heatmap. Labels for each SCINS measure are displayed on the left. Box plots underneath depict the distribution of $S_{\text{AbCNA}}$, $S_{\text{CnLOH}}$, and $S_{\text{HiCnLOH}}$ across the three clusters, identifying for each dataset a Lo-SCINS (green arm of dendrogram), a Hi-AiCNA (red arm of dendrogram), and a Hi-CnLOH (blue arm of dendrogram) group. C and D, volcano plots of the log fold change of expression for all genes (rows) across the dataset. Heatmap colors represent mean-centered log expression values. The genes in each heatmap are ordered from top to bottom by ascending (rows) across the dataset. Heatmap colors represent mean-centered log expression values. The genes in each heatmap are ordered from top to bottom by ascending $q$-value, and then by the descending $T$-statistic value of the SAM method. The top panel of heatmaps shows the genes that are predominantly expressed in the Hi-AiCNA cluster. The middle panel of heatmaps shows genes differentially upregulated in the Hi-CnLOH cluster, and the bottom panel shows those specifically upregulated in the Lo-SCINS cluster. E, F, and G, Venn diagrams showing the overlap between the differentially expressed genes (FDR $Q < 0.1$, SAM) from the KCL and METABRIC TNBC data for the Hi-AiCNA clusters (red shading), Hi-CnLOH clusters (blue shading), and the Lo-SCINS clusters (green shading). H, table showing the five genes belonging to the Hi-AiCNA list in both KCL and METABRIC, including HORMAD1.
those involving TP53 mutation and RB1 loss, and high HORMAD1 expression (Supplementary Fig. S6A–S6J) prompted us to conduct functional experiments to test whether HORMAD1 overexpression could cause chromosomal instability.

HORMAD1 Is Expressed in the Nucleus of Breast Cancer Cells in Both Cell Lines and Tumors

We turned to cellular models of breast cancer to provide a tractable system to investigate the consequences of HORMAD1 expression and whether the relationship with $S_{\text{AiCNa}}$ was causative. We first confirmed a relationship between HORMAD1 expression and $S_{\text{Ai}}$ in a panel of 23 breast cancer cell lines for which gene expression was available (Fig. 4A). The bimodal expression pattern in TNBC was replicated at the protein level in cellular models where HORMAD1 protein was expressed in five of the nine ER and HER2-negative cell lines examined (HCC70, HCC1143, MDA MB436, HCC38, and CAL51) with levels showing good concordance with gene expression data (Fig. 4B).

Furthermore, no expression of HORMAD1 protein was seen in two nontransformed mammary epithelial cells, MCF10A and HMEC (Fig. 4B). In meiotic cells, HORMAD1 localizes to the nucleus (17) and can recognize aberrant DNA structures, possibly due to its chromatin-associated HORMA domain (30). Subcellular fractionation of cells in triple-negative HCC1143 breast cancer cells confirmed the presence of HORMAD1 in the nuclear fraction (Fig. 4C), where it appears to be constitutively associated with chromatin in a DNA damage–independent manner (Fig. 4D).

Induction of DNA damage by irradiation (IR) or hydroxyurea (HU) did not induce a detectable change in HORMAD1 localization by IF (Supplementary Fig. S7B). A
similar nuclear staining pattern was also observed by immunocytochemistry in the HORMAD1-expressing cell lines HCC38 and HCC70 (Supplementary Fig. S8A). Interrogation of tissue microarrays confirmed nuclear localization of HORMAD1 in primary TNBC (Supplementary Fig. S8A). To quantify HORMAD1 protein levels in primary tumors, we carried out protein extractions and Western blot analyses (Fig. 4F and Supplementary Fig. S8B). HORMAD1 protein expression was detectable in 13 of 15 tumors identified as having high HORMAD1 by gene expression. Protein expression in these tumors was at comparable levels with that in HORMAD1-expressing TNBC cell line models (Fig. 4F). As anticipated, tumors in the low HORMAD1 transcript group had no detectable protein (Fig. 4F).

**Figure 4.** HORMAD1 expression in cell lines and primary tumor tissue. A, correlation of the composite S_{AI} measure (vertical axis) with HORMAD1 expression (horizontal axis) in 26 breast cell lines (red, TNBC; black, non-TNBC). BRCA1 status of these samples is indicated by triangles (mutated) or circles (wild-type). The Spearman rank correlation coefficient and P value are shown. B, Western blot analysis of HORMAD1 in breast cancer and non-transformed cell lines, cell lysates were loaded in order of HORMAD1 gene expression, with highest expression on the left. Values below blots represent quantification of HORMAD1 bands normalized to actin. C, subcellular fractionation of proteins in HCC1143 cells. D, fractionation of nuclear proteins into nuclear soluble and insoluble fractions before and after DNA damage induction by HU, top, and IR, bottom. Induction of DNA damage was validated by probing for γ-H2AX. E, IF of HORMAD1 localization in breast cancer cell lines (red, HORMAD1; blue, nuclei). F, quantification of HORMAD1 protein levels by Western blot analysis in primary tumor samples and cell lines. Tumors are grouped into low and high HORMAD1 based on gene expression values using the bimodal cutoff value of 5.9. Cell lines are color coded as follows: blue, HCC1143; red, SUM159-HORMAD1-V5; green, MDA-MB-436; gray, SUM159. In tumor samples, the central bar represents the mean protein level across all samples in that group, whereas in the cell lines, the mean of HORMAD1-positive samples only is shown.

**HORMAD1 Expression Drives S_{AI} and Chromosomal Instability**

In breast cancer cell lines, HORMAD1 expression was significantly associated with S_{AI} (r_s = 0.46; P = 0.019; Fig. 4A). Although we had established an association between HORMAD1 expression and genomic instability in triple-negative breast tumors and cell line models, it was not clear at this point whether elevated HORMAD1 expression drove genomic instability or was a consequence. To this end, we created stable populations of HORMAD1- and control LacZ-expressing SUM159 cells, a triple-negative cell line model with relatively low levels of genomic instability (31) and low HORMAD1 expression. Expression of HORMAD1 or LacZ did not change the growth rate of SUM159 cells (Supplementary Fig. S9A).
In order to test whether HORMAD1 expression induced the similar genome scarring patterns as those associated with elevated HORMAD1 expression in primary TNBCs, we compared the SNP6.0 array profiles and SCINS from HORMAD1- and LacZ-expressing populations of cells grown under the same conditions for the same period of time over two independent experiments. We found both $S_{SCINS}$ and $S_{Ai}$ to be higher in the HORMAD1-expressing SUM159-HORMAD1-V5 cells compared with the control-treated SUM159-LacZ-V5 cells, with an average fold change in these scar measures of 4 and 1.5, respectively (Fig. 5A). No increase in $S_{G2A}$ was observed in SUM159-HORMAD1-V5 cells compared with SUM159-LacZ-V5 cells (Fig. 5A). This observation was consistent with the hypothesis that HORMAD1 might be capable of driving the generation of specific genomic scars, specifically, allelic imbalances accompanied by copy-number change.

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Figure 5. (Continued) F, the effect of HORMAD1 knockdown on DR-GFP HR reporter activity in the HORMAD1-expressing cell lines, HCC1143 and MDA-MB-436. HR activity was assessed by quantifying GFP-positive cells 72 hours after cotransfection of DR-GFP and pCBASce. Bar plots, mean, and SEM of three independent experiments. Statistical significance was assessed by a Student t test. The mean absolute percentage of GFP-positive cells in each condition is stated in green above bars. G, SUM159-LacZ-V5 and SUM159-HORMAD1-V5 cells were assessed for the presence of spontaneous nuclear γH2AX foci. Data shown are representative of triplicate experiments. Data, number of γH2AX foci in individual nuclei; bars, mean and SD for each condition. Statistical significance of the mean number of foci between each group was assessed by a Student t test. H, IR (left) and HU (right)-induced RAD51 focus formation in SUM159-LacZ-V5 or SUM159-HORMAD1-V5 cells. Data shown are representative of triplicate experiments. Data, number of RAD51 foci in individual nuclei; bars, mean and SD for each condition. Statistical significance of the mean number of foci between each group was assessed by a Student t test. I, IR (left) and hydroxyurea (right)-induced 53BP1 focus formation in SUM159-LacZ-V5 or SUM159-HORMAD1-V5 cells. Data, number of 53BP1 foci in individual nuclei; bars, mean and SD for each condition. Statistical significance of the mean number of foci between each group was assessed by a Student t test. J, the EJ5 NHEJ reporter vector was used to assess NHEJ activity in SUM159 cells stably expressing HORMAD1 (left) and HORMAD1 knockdown HCC1143 cells (right). NHEJ activity is presented as a percentage compared with the control. Bars, mean of three independent transfections.

Metaphase spreads were prepared from SUM159-HORMAD1-V5 and SUM159-LacZ-V5 populations, and chromosomal abnormalities were counted (Fig. 5B). Overall, SUM159-HORMAD1-V5 cells had a 4-fold greater number of structural chromosomal abnormalities than SUM159-LacZ-V5 cells (P = 0.002), with a significant increase in acentric fragments and chromatid loops (P = 0.038 and P = 0.021, respectively; Fig. 5B).

Aberrant nuclear structures, such as micronuclei (MN), nuclear buds (NBUD), and nucleoplasmic bridges (NPB), are biomarkers of induced genotoxic events and chromosomal instability (32). We therefore assessed SUM159-HORMAD1-V5 and SUM159-LacZ-V5 populations for the presence of these nuclear anomalies (Fig. 5C). Overall, SUM159-HORMAD1-V5 cells exhibited a 3.25-fold increase in MN, NBUD, and NPB compared with SUM159-LacZ-V5 cells. Individually, fold increases of 2.2, 2.7, and 2 were observed for MN, NBUD, and NPB, respectively (Fig. 5C).

HORMAD1 is involved in many aspects of meiotic recombination, in which RAD51-dependent equal sister chromatid recombination is inhibited in favor of DMC1-mediated recombination and crossover formation with the homologous chromosome (33–35). For this reason, we examined whether inappropriate HORMAD1 expression in a mitotic cell context might suppress conservative HR in TNBC cell line models. We
used the DR-GFP assay to assess conservative HR of an I-SceI endonuclease-induced double-strand break (36). Overexpression of HORMAD1 in this system caused a 55% reduction in HR in SUM159 (HORMAD1-negative) cells (Fig. 5D). Overexpression of HORMAD1 in the HORMAD1 low expressing cell line CALS1 also caused a 36% decrease in HR activity (Fig. 5E and Supplementary Fig. S9B). In contrast, siRNA-mediated knockdown of HORMAD1 in HCC1143 and MDA-MB-436 cells (both HORMAD1-positive lines) caused an increase in HR (100% and 55% increases, respectively; Fig. 5F and Supplementary Fig. S9C). Ectopic expression of HORMAD1 in mouse embryonic stem (ES) cells with an integrated DR-GFP reporter also led to reduced HR levels (Supplementary Fig. S9D). These data suggest that HORMAD1 is capable of regulating HR activity in established breast cancer cells but also in nontransformed cells. To assess whether HORMAD1-expressing cells show failure to repair spontaneous DNA double-strand breaks, we assessed the number of spontaneous γH2AX nuclear foci and found SUM159-HORMAD1-V5 cells to have more than twice the mean number of γH2AX nuclear foci, a biomarker of double-strand breaks, compared with SUM159-LacZ-V5 cells (fold increases of 7.5 and 3.3, respectively; Fig. 5G and Supplementary Fig. S9E). To further test the hypothesis that HORMAD1 modulates RAD51-dependent HR and double-strand break repair, we measured hydroxyurea and IR-induced RAD51 focus formation, a biomarker of HR, and observed a significant reduction in the number of DNA damage–induced RAD51 foci in SUM159-HORMAD1-V5 cells when compared with SUM159-LacZ-V5 cells (Fig. 5H and Supplementary Fig. S9F). Transient expression of HORMAD1 in this assay caused a modest reduction in SUM159 cell growth (80%; P = 0.05) and a statistically significant increase (P = 0.01) in the proportion of cells in S-phase (37%) compared with empty vector–transfected SUM159 (27.8%; Supplementary Fig. S10A and S10B). Alterations in cell-cycle distribution, specifically a reduction in the number of S-phase cells, can result in an apparent reduction in HR activity. However, because transient HORMAD1 expression causes an increase in the proportion of cells in S-phase, this is unlikely to be an explanation for the reduction in HR activity that we observe (Supplementary Fig. S10B). Conversely, siRNA-mediated knockdown of HORMAD1 in MDA-MB-436 and HCC1143 cells caused a reduction in the percentage of cells in S-phase (Supplementary Fig. S10C), and is equally unlikely to explain the increase in HR activity that we observe in these cells after HORMAD1 knockdown. In fact, we suggest that HORMAD1-induced impairment of the repair of replication-associated DNA damage is likely to cause an accumulation of cells in S-phase.

Having observed both an impairment of HR and an increase in specific forms of chromosomal instability in HORMAD1-overexpressing cells, we asked whether such cells exhibited increased levels of NHEJ repair, a DNA double-strand break process that is commonly upregulated in response to HR deficiency. NHEJ activity was assessed by measuring the induction of 53BP1 focus formation by both IR and hydroxyurea (Fig. S1 and Supplementary Fig. S1A) and the repair of I-SceI endonuclease-induced double-strand breaks in the previously validated EJ5 NHEJ GFP reporter assay (Fig. S5); ref. 37). In both assays, SUM159-HORMAD1-V5 cells showed significantly increased levels of NHEJ activity compared with SUM159-LacZ-V5 cells (Fig. S1–J). A similar increase in NHEJ activity was observed with transient transfection of HORMAD1, suggesting that expression of HORMAD1 had a direct, acute effect on NHEJ activity (Supplementary Fig. S11B and S11C). Furthermore, despite long-term exposure to endogenous HORMAD1 expression, knockdown of HORMAD1 in HCC1143 reduced EJ5 NHEJ GFP reporter–measured NHEJ (Fig. S5) and damage-induced 53BP1 focus formation (Supplementary Fig. S12A), with a modest nonsignificant increase in nuclear RAD51 foci also observed (Supplementary Fig. S12B).

**HORMAD1 Overexpression Drives Sensitivity to HR Defect–Targeting Drugs in TNBC**

Given the association between high S_{AiCNA}, in particular high S_{AiCNA, and platinum salt sensitivity (Fig. 1 and Supplementary Fig. S13A–S13F), along with the established observation that HR-deficient cells show increased sensitivity to platinum salts and PARP inhibitors (38), we sought to examine whether there was a causative role for HORMAD1 in increasing platinum agent sensitivity. As such, we investigated the effect of HORMAD1 overexpression on the sensitivity of SUM159 and CALS1 cells to cisplatin. Consistent with the effect in the DR-GFP and RAD51 HR assays, HORMAD1 overexpression increased sensitivity of both SUM159 and CALS1 cell lines to cisplatin, with HORMAD1 expression decreasing cisplatin SF_{10} from 10.8 to 4.4 μmol/L in SUM159 (P < 0.0001), and from 7.4 to 3.3 μmol/L in CALS1 (P = 0.003; Fig. 6A). Next, we assessed whether breast tumor cell line models with elevated HORMAD1 expression (Fig. 4B) exhibited sensitivity to potent small-molecule PARP inhibitors, which have previously been shown to selectively target tumor cells with BRCA1 or BRCA2 defects (39). Using dose–response survival experiments in cells exposed to the clinical PARP1/2 inhibitor olaparib (AZ/KuDOS; ref. 39), we found that MDA-MB-436, HCC1143, HCC70, and CALS1 models, each of which expressed elevated levels of HORMAD1, all exhibited a moderate level of sensitivity. This was in contrast with the relative olaparib insensitivity observed in HORMAD1-deficient HSS787T, BT20, MDA-MB-231, SKBR3, and SUM159 models (Fig. 6B and C). To test whether elevated HORMAD1 expression could directly drive PARP inhibitor sensitivity, we examined the PARP inhibitor sensitivity of SUM159-HORMAD1-V5 and SUM159-LacZ-V5 populations. SUM159-HORMAD1-V5 cells showed a significantly greater sensitivity to olaparib (left, P = 0.0001; Fig. 6D). To eliminate the possibility that this observation might be specific to olaparib, we assessed the sensitivity of these cells to a novel, hyperpotent clinical PARP inhibitor, BMN673 (right, P = 0.0001; Fig. 6D; ref. 40). The PARP inhibitor sensitization, resulting from overexpression of HORMAD1, although significant, was relatively modest compared with that produced by BRCA2 mutation (Fig. 6D). This is consistent with the moderate reduction in HR, sufficient to induce genomic instability without profound cell-cycle arrest, seen in previously described assays (Fig. 5D, E, and H) and indicates that HORMAD1 expression partially rather than completely ablates HR competency.

We looked at the reciprocal effect of HORMAD1 siRNA on the sensitivity of the high-HORMAD1 cell line HCC1143 to cisplatin and the PARP inhibitor BMN673 (Supplementary Fig. S14A and S14B). Despite long-term exposure to endogenous HORMAD1 expression and potential adaptation, we observed the anticipated modest but statistically significant...
**Figure 6.** HORMAD1 expression directly induces sensitivity, and contributes as an indicator of response to HR defect-targeting agents. **A**, cisplatin survival curves of SUM159 (left) and CAL51 (right) cells transfected with HORMAD1 expression vector. Data shown are the mean and SEM from three experiments. Statistical significance was assessed using ANOVA. Error bars, SEM from three replica experiments. **B**, dose–response olaparib survival curves for a panel of breast tumor cell lines. Cells were plated in 6-well plates and constantly exposed to olaparib for 2 weeks, at which point survival was estimated. As a control for olaparib sensitivity, the BRCA1 mutant DLD1 or a control LacZ cDNA, which were plated in 6-well plates and exposed to either the clinical PARP inhibitor olaparib (left) or the clinical PARP inhibitor BMN673 (right). DLD1 BRCA2 mt and DLD1 BRCA2 wt cells are included as a positive control for PARP inhibitor sensitization. Statistical significance was assessed using ANOVA. Error bars, SEM from three replica experiments. **C**, bar chart of olaparib SF50 values interpolated from [Cisplatin] (mol/L) for a panel of breast tumor cell lines. Cells were transfected with HORMAD1 expression vector. Data shown are the mean and SEM from three experiments. Statistical significance was assessed using ANOVA. Error bars, SEM from three replica experiments. **D**, bar chart of olaparib SF50 values interpolated from [Olaparib] (mol/L) for a panel of breast tumor cell lines. Cells were transfected with HORMAD1 expression vector. Data shown are the mean and SEM from three experiments. Statistical significance was assessed using ANOVA. Error bars, SEM from three replica experiments. **E**, HORMAD1 expression and BRCA1 mutation, and those with at least one of these gene dysregulations was optimally accurate. Individual and combined predictors of platinum-based chemotherapeutic response using the whole PrECOG TNBC dataset are shown in yellow and blue, respectively. ROC analyses using dichotomized HORMAD1, S_{AiCNA}, S_{CnLOH}, and S_{Ai} as predictors were used in the BRCA1/2 wild-type subpopulation of the PrECOG dataset (bars in orange), and balanced accuracy computed as before.
reduction in sensitivity to BMN673 following HORMAD1 knockdown, consistent with the effects observed on NHEJ, 53BP1, and RAD51 focus formation (Fig. 5) and Supplementary Fig S12). However, no effect on cisplatin sensitivity in this cell line was observed, indicating that although expression of HORMAD1 is sufficient to induce platinum agent sensitivity, it is not always necessary. Many DNA repair defects contribute to cisplatin sensitivity, and HORMAD1 knockdown in a long-term HORMAD1-adapted cell line is unlikely to significantly change response to a chemotherapy drug acting through several mechanisms. In contrast, PARP inhibition has a more selective mechanism of action, more directly related to its specific targeting of HR dysfunction and potentially more sensitivity to HORMAD1 expression levels.

Having demonstrated in cell line models that HORMAD1 is capable of driving sensitivity to HR defect–targeting drugs, we examined the applicability of HORMAD1 expression and the three allelic imbalance–based SCINS, when measured in pretreatment samples, to predict therapeutic response in the trial data (NCT#00813956; ref. 26). This single-arm trial treated patients with primary TNBC or BRCA1/2 mutation–associated breast cancer with the combination of gemcitabine and carboplatin, and the investigational agent iniparib, which has no validated mechanism of action and is considered unlikely to have had any dominant effect on tumor response based on randomized trial data comparing gemcitabine and carboplatin alone with the addition of iniparib (41). PreECOG TNBCs with overlapping SNP and gene expression data were dichotomized into high (n = 29) and low (n = 30) HORMAD1–expressing cases based on the bimodality of gene expression, resulting in 43% and 69% responders in the HORMAD1–low and HORMAD1–high groups, respectively (Fig. 6E). Receiver-operating characteristic (ROC) analysis revealed BRCA1/2 mutation status to have high specificity for treatment response but much poorer sensitivity than dichotomized HORMAD1 expression and allelic imbalance–based SCINS (Supplementary Fig. S15). This indicates that although patients with mutations in BRCA1/2 carry a very high probability of response, a spectrum of response is observed in those with sporadic TNBCs, many of which are BRCA1/2 wild-type (4, 5). We therefore asked whether the performance of BRCA1/2 mutation status in predicting platinum response could be enhanced by supplementation with HORMAD1 expression or allelic imbalance–based SCINS. We used ROC analysis to define cutoffs that maximized the balanced accuracy of a predictor and found that combining HORMAD1 expression or the allelic imbalance–based SCINS with BRCA1/2 mutation status led to an improvement in accuracy over that for each predictor alone (Fig. 6F, yellow and blue bars). Given that HORMAD1 might be informative of platinum response independently of BRCA1/2 mutation, we looked within the BRCA1/2 wild-type subpopulation of the PreECOG dataset after first confirming that there was no substantial shift in the distribution of or cutoff for HORMAD1 bimodal expression (Supplementary Fig. S16A and S16B). We found the balanced accuracy to be either maintained (HORMAD1) or improved (allelic imbalance–based SCINS) within this subset of TNBCs when compared with the accuracies of the single markers in the whole population (Fig. 6F, orange bars). These findings suggest that HORMAD1 and the allelic imbalance–based SCINS may find utility either within a BRCA1/2–proficient setting or in combination with BRCA1/2 mutation testing.

DISCUSSION

TNBCs are characterized by high levels of chromosomal instability, and therapies leveraging on DNA repair defects have emerged with varying degrees of success. In this study, we have developed an approach to capture diverse genomic patterns in TNBC, which may report the activity of different combinations of mutational mechanisms. One such chromosomal aberration, S_{AiCNA}, was associated with platinum sensitivity in neo-adjuvantly treated TNBC and HGSC, even among BRCA1/2–proficient cases. Transcriptional profiling of TNBCs grouped on the basis of different genomic aberrations identified the misexpression of the meiotic cancer testis antigen HORMAD1 as a novel driver of genome instability. Consistent with the notion of S_{AiCNA} as a measure of defective HR, HORMAD1 negatively regulated HR activity in cell lines and led to the generation of MN and structural chromosomal aberrations. Thus, we provide the first direct experimental evidence of the causative role of a gene whose normal function is restricted to the inhibition of conservative meiotic sister chromatid HR and creation of genetic diversity, in the generation of potentially clinically relevant genomic scars of HR deficiency.

Mutational signatures and genomic scars have been shown to represent reasonable surrogates of BRCA1/2 mutation and platinum salt sensitivity, and are currently being tested as companion diagnostics in prospective PARP inhibitor trials for HGSC (NCT#01891344; refs. 9–12). By generating multiple scores, we expanded on these approaches to uncover the granularity of genomic alterations present in TNBC. We show that each scar, although largely independent of the others, had a consistent distribution across four independent TNBC cohorts. The most prevalent and informative genomic scar for platinum sensitivity was S_{AiCNA}, pointing to the frequent activation of error-prone DNA repair processes in these tumors.

Taking account of the mitotic function of HORMAD1 in promoting HR with non–sister chromatid templates, we sought evidence of upregulation of nonconservative HR in SNP microarray data by using the number of AiCNAs associated with copy-number loss between low copy-number repeats as a surrogate measure of intrachromosomal nonallelic HR. Given the limitations of SNP arrays to report products of the varied forms of nonconservative homology–directed repair accurately, the results are inconclusive (data not shown). Therefore, we cannot currently exclude the possibility that HORMAD1, through favoring the use of non–sister chromatid homology templates, also increases the use of non–conservative recombination between nonallelic homologous templates (33) as an additional driver of AiCNAs across the genome (Fig. 7). The absence of an increase in CnLOH associated with expression of HORMAD1 cDNA in our SUM159 model (Fig. 5A) suggests that HORMAD1 expression in this specific context does not increase allelic forms of interhomolog recombination. However, further study in a wider range of cellular contexts is warranted.

Studies in mice have identified four major mitotic functions for HORMAD1 (Fig. 7A; refs. 18, 42, 43). However,
**Figure 7.** Mechanism of HORMAD1-induced genomic instability. A, HORMAD1 is an essential meiotic protein that plays at least four important roles in meiosis: (i) it supports SPO11 to induce double-strand breaks; (ii) it acts alongside Ataxia Telangiectasia and Rad3-related (ATR) as part of the meiotic silencing of unsynapsed chromosomes pathway; (iii) it is essential for the formation of the synaptonemal complex, and (iv) it contributes to the maintenance of interhomolog bias in template choice for recombinational repair of double-strand breaks. B, of these, its role in maintaining interhomolog bias is particularly relevant to its role in genomic instability in TNBC. HORMAD1 favors interhomolog repair by inhibiting RAD51, which functions in intersister chromatid repair, while promoting DMC1, an interhomolog-acting recombinase. This bias ensures chiasma formation, which is essential for normal segregation of chromosomes at meiosis. When HORMAD1 is expressed out of context in mitotic cells, this function of HORMAD1 induces genomic instability by inhibiting RAD51 activity and thus normal HR. This leads to an increased reliance on error-prone NHEJ resulting in aberrations, including AiCNA. In addition, HORMAD1 may also promote nonconservative allelic or repetitive sequence driven HR.

Our understanding of its activity in mitotic cells is currently limited (44). During meiosis, cells undergo a wave of double-strand break induction catalyzed by the topoisomerase SPO11, leaving stretches of single-stranded DNA to which RAD51 or DMC1 associate. DMC1 is a meiosis-specific recombinase that promotes homology-directed repair between homologous chromosomes. These events and their associated crossovers are vital for both the creation of genetic diversity and the generation of chiasmata between homologous chromosomes, which ensure accurate meiotic chromosome segregation (18, 33). HORMAD1 supports the activity of both SPO11 and DMC1. Although higher expression of SPO11 and DMC1 has previously been reported in melanoma and lymphoma, respectively (45), we found no evidence for...
their expression in TNBC (data not shown). In meiosis, in contrast with somatic cells, most double-strand breaks are repaired via homology-directed repair using the homologous chromosome rather than the sister chromatid template with the aim of creating genetic diversity (22). HORMAD1 and its orthologs, Hap1 and Asy1, support this interhomolog bias as part of the barrier to sister chromatid repair, by inhibiting RAD51 engagement with sister chromatids (Fig. 7B; refs. 35, 43, 46). Our observations of reduced HR and RAD51 foci formation in breast cell lines point to an out-of-context activation of the barrier to sister chromatid repair occurring upon HORMAD1 expression in mitotic cells. In meiosis, this process has been shown to involve the inhibition of RAD54 (35, 46), which, in mouse embryonic stem cells, is essential for RAD51 focus formation after DNA damage (47). Low levels of many RAD54 paralogs in TNBC might suggest that HORMAD1 inhibits HR and RAD51 loading via a similar mechanism. Although its causative relationship with genomic instability and SAcNA suggests further study to fully define its mechanism (Fig. 7B), the abnormal expression of this germ cell protein supports the hypothesis of “meioticosis,” low-level expression of meiotic components in mitotic cells that leads to genomic instability in cancer (48). Along with HORMAD1, our analysis identified higher transcript levels of other meiotic genes, such as PSMC3IP in TNBC, in high SAcNA tumors. Overexpression of truncating mutants of PSMC3IP involved in recombination was previously found to inhibit RAD51 focus formation in cell lines (49). In addition, SYCP2L, which encodes a protein that is engaged in the synaptonemal complex, was of higher abundance in genomically unstable HGSCs. Thus, our data add to evidence suggesting a potentially wider role for abnormal expression of meiotic genes in the causation of genomic instability in tumors.

In this article, we have shown that the study of the whole-genome allelic landscape in TNBCs reveals significant heterogeneity between tumors in the genome instability mechanisms that correlate with sensitivity to platinum salts and PARP inhibitors that are currently being considered for unselected patients with TNBC. In contrast with previous studies identifying genomic scars of HR defects, we have both identified and functionally validated a novel driver of impaired RAD51-dependent HR, and upregulated NHEJ associated with AiCNA-related genome instability and HR defect–targeting drug sensitivity. The degree of impairment of HR and consequent sensitization to platinum salts and PARP inhibitors, caused by inappropriate HORMAD1 expression, is more modest than that induced by loss-of-function BRCA2, the key regulator of RAD51 in mitotic cells. However, HORMAD1-induced upregulation of nonconservative DNA repair, coupled with a lack of significant impairment of cell proliferation, appears sufficient to cause acquisition of increased genomic instability in affected cell populations, suggesting a significant role in mutagenesis and the etiology of basal-like TNBC.

The bimodal expression of HORMAD1 in TNBC, along with its restricted expression in normal tissue, suggests that further examination of this gene as a biomarker component in trials of therapies that target HR deficiency in this tumor subtype is feasible and warranted. Although further evidence from multiple independent datasets and randomized therapy trials is required, our initial findings in the PrECOG0105 trial suggest that an AiCNA measure, HORMAD1 expression and BRCA1/2 mutation status, may have value in patients with TNBC as a composite predictive biomarker for platinum sensitivity.

METHODS

Clinical Sample Data

A discovery dataset comprising 142 fresh-frozen, macrodissected primary invasive TNBCs was obtained from King’s Health Partners Cancer Biobank (London, United Kingdom; referred to as KCL data). A detailed description of clinicopathologic features has been published previously (50) and is provided in Supplementary Table S1. From these, we were able to extract DNA, generate SNP6.0 data (Affymetrix), and reconstruct copy-number profiles for 111 samples. Raw data from the gene expression microarray and SNP6.0 array experiments are available for download at under the accession numbers GSE40267 (http://www.ncbi.nlm.nih.gov/geo/) and E-MTAB-2626 (http://www.ebi.ac.uk/arrayexpress/), respectively. Further details on the samples and processing of the METABRIC, TCGA TNBC, TCGA HGSC, and PrECOG TNBC cohorts are provided in the Supplementary Methods.

Cell Lines and Cell Line Data

Public SNP6.0 microarray data for cell lines were obtained from the Sanger Cell Lines Project. For a further 18 breast cell lines, DNA was isolated and SNP6.0 copy-number profiles were obtained via Atlas Biolabs GmbH. All cell lines were cultured according to the supplier's recommendations, and were characterized and authenticated by short tandem repeat (STR) profiling (27). Gene expression profiling and processing for cell lines has been described previously (27). The BRCA1/2 mutation, ER, PR, and HER2 statuses of cell lines are listed in Supplementary Table S1. Cell lines were authenticated by STR profiling and used only up to 30 passages from authentication.

DNA Copy-Number Analysis

For the PrECOG and TCGA (TNBC and HGSC) datasets, matched blood samples were used as reference, whereas for the KCL, METABRIC, and cell line data, HapMap270 samples provided by Affymetrix were taken as the reference genome. For SNP microarray data from the polyclonal SUM159 samples transfected with either HORMAD1 or LacZ, data from the SUM159 ancestor were used as reference. For the KCL, METABRIC, PrECOG, TCGA TNBC, and cell line data, allele-specific copy-number profiling was performed with the Tumor Aberration Prediction Suite (7), whereas for the TCGA HGSC dataset, allelic copy-number construction was implemented using allele-specific copy-number analysis of tumors (ASCAT; ref. 8). Further details are described in the Supplementary Methods.

Scores of Chromosomal Instability Scarring

Segments of allele-specific copy-number profiles were categorized into one of three nonoverlapping types—AiCNAs, AbCNAs, and CnLOH—forming the basis for SCINS (Supplementary Table S2). In brief, AiCNA was determined by calculating the proportion of the genome consisting of CnLOH segments and saving those segments that encompassed a whole chromosome. Next, the number of AiCNA segments greater than or equal to 8 Mbp in length but less than the length of a whole chromosome was counted, and this number was multiplied by the proportion (excluding whole chromosome segments) to give the AiCNA measure (SAcNA). For CnLOH, the proportion of the genome consisting of CnLOH segments was calculated only if the segment was greater than or equal to 4 Mbp in length but less than the length of a whole chromosome. Similar to SAcNA, the measure of CnLOH segments (SCnLOH) was calculated by multiplying the proportion obtained by the number of segments. For SAcNA, the measure of AbCNA segments, the number of AbCNA
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segments greater than or equal to 8 Mbp in length was counted. In addition, one summary measurement was established, \( S_{\text{DNA}} \), by summing \( S_{\text{SCINS}} \) and \( S_{\text{CnLOH}} \), thereby capturing all allelic imbalance events. A document describing SCINS is provided in the Supplementary Data. \( N_{\text{All}} \) was calculated using a code obtained from ref. 10, whereas a version of the Myriad HRD score, which we term \( S_{\text{DNA}} \), was based on details given in ref. 9.

Kolmogorov–Smirnov tests were used to investigate differences in the distribution of (i) the distance between adjacent aberration breakpoints and (ii) SCINS among different cohorts. Wilcoxon rank-sum tests were used to test the association between SCINS and chemotherapy responders/nonresponders. The Fisher exact test was used to test for independence between groups. The relationships among genomic scarring scores, and also between these scores and gene expression, were measured by the Spearman rank correlation. The robustness of hierarchical clustering with the Ward criterion was evaluated using the pvclust R package (51). To test the ability of HORMAD1 expression to predict platinum-based chemotherapeutic response in PrECOG TNBCs, logistic regression analysis was performed. Optimally accurate cutoffs were established through ROC analyses and the balanced accuracy calculated as (sensitivity + specificity)/2. All \( P \) values are two-tailed, and those with values less than 0.05 was considered significant unless otherwise stated. All microarray and statistical analyses were performed in the R statistical language environment 2.14.1 (52) using several Bioconductor packages.

Gene Expression Analysis

SAM analysis was used to assess differential gene expression between SCINS-defined clusters using the Benjamini–Hochberg method to correct for multiple testing. Details about the meta-analysis, gene signature analysis, and grouping of tumors by HORMAD1 expression are provided in the Supplementary Methods.

Expression Vectors

The HORMAD1 (NM_032132) expression vector pEZ-M67 (GeneCopoeia) was used for transient expression of HORMAD1. For stable expression, the HORMAD1-V5 lentiviral vector was used. This was produced by cloning the coding sequence from pEZ-M67 into the pLenti6/V5-DEST Gateway vector (Life Technologies). The pLenti6.2/V5-GW/LacZ vector (Life Technologies) was used as a negative control for the effect of lentiviral infection and selection on cells. The HORMAD1 cDNA from pEZ-M67 was additionally cloned into the pCAGGS vector for use in mouse ES cell experiments.

siRNA-Mediated Silencing

Human Silencer Select siRNAs included a nontargeting negative control siRNA (ID: 4390843) and HORMAD1 targeting siRNA (s38456; Ambion, Life Technologies).

Antibodies

Primary antibodies used were HORMAD1 [HPA037850; Sigma-Aldrich; 1:200 (IF), 1:500 (WB)], β-actin (A5316; Sigma-Aldrich; 1:20,000), Histone-H3 (9715; Cell Signaling Technology; 1:1,000), CENP-A (2186; Cell Signaling Technology; 1:1,000), γH2AX [Ab22551; Abcam; 1:4,000 (IF), 1:1,000 (WB)], RADS1 (sc-8349; Santa Cruz Bio-technology; 1:500), GAPDH (D16H11; Cell Signaling Technology; 1:500), and 53BP1 (Clone BP13; Millipore; 1:500). Secondary antibodies used were Alexa Fluor 488 goat anti-mouse IgG, Alexa Fluor 555 donkey anti-rabbit IgG (Life Technologies; 1:4,000), or anti-mouse/rabbit horseradish peroxidase (HRP) conjugate (GE; 1:20,000).

Protein Fractionation

Cells were lysed in fractionation buffer [250 mmol/L sucrose, 20 mmol/L HEPES (7.4), 10 mmol/L KCl, 1.5 mmol/L MgCl₂, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L DTT, and protease inhibitors] to produce cytoplasmic fraction. Whole nuclear fractions were produced using fractionation buffer supplemented with 10% glycerol and 0.1% SDS. Nuclear soluble and insoluble fractions were produced by resuspending the nuclear pellet in 20 mmol/L HEPES, pH 8.0; 1.5 mmol/L MgCl₂, 25% glycerol; 420 mmol/L NaCl; 0.2 mmol/L EDTA; 1 mmol/L DTT; and 0.5 mmol/L phenylmethylsulfonylfluoride (PMSF) and separated by centrifugation.

Preparation of Protein Lysates from Primary Tumors

Tumor protein lysates were prepared from paraffin-embedded tumor material using Laemmli buffer. The volume of buffer used for each sample was varied depending on cellularity and tumor surface area.

Metaphase Spreads

The mitotic index was increased by treatment with colcemid, and cell pellets were resuspended in 0.075 mol/L KCl and fixed in ice-cold alcohol fixative (3:1 methanol and acetic acid). Spreads were dropped at 25°C and 50% humidity, air-dried, and stained with Leishman’s stain (in 1:5 buffer solution at pH 6.8). Metaphase spreads and aberrations were validated independently by a cytogeneticist.

DR-GFP Homologous Recombination Assay

Cells were seeded into 6-well plates and transfected with 3 μg of the 1-Ciel expression plasmid (pCBASEc) and 2 μg of the DR-GFP reporter using FuGENE 6 transfection reagent (Promega). GFP fluorescence was assessed by FACS 72 hours after transfection. For HORMAD1 overexpression assays, 5 μg of HORMAD1 plasmid was transfected into cells using FuGENE 6 (Promega), 24 hours prior to I-SceI/DR-GFP transfection. For knockdown assays, cells were transfected with siRNA 24 hours prior to I-SceI/DR-GFP transfection. DR-GFP assays in mouse ES cells were carried out as previously described (36).

EJ5 NHEJ Assays

Transient transfection of the EJ5 NHEJ vector was used as a crude measure of total NHEJ (37). Cells were seeded and transfected with the EJ5 NHEJ and 1-Ciel plasmid and assessed for GFP fluorescence 72 hours later by FACS.

Immunofluorescence and Micronuclei Assay

Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich) and permeabilized in 0.5% Triton X-100 (Sigma-Aldrich). For MN assessment, cells were stained with 0.2 mg/mL Hoechst (Promega). Nuclei in 25 random fields (>500 cells) were assessed for the presence of aberrant nuclear structures. For IF, cells were blocked in 20% BSA in 0.1% Triton/PBS prior to incubation with antibody. Cells were counterstained with 0.2 mg/mL Hoechst. To assess foci formation, random fields were taken with a fluorescent microscope and the number of foci assessed by counting >50 cells.

Cisplatin Sensitivity Assays

Cells were seeded into a 96-well plate and transfected with HORMAD1 or empty expression vector using FuGENE 6 (Promega). Cisplatin was added the following day and cell viability assessed 5 days later using CellTiter-Blue (Promega). Readings were normalized to vehicle treated to give percentage survival.

PARP Inhibitor Sensitivity Assays

Olaparib was purchased from SelleckChem. BMN673 (40) was the kind gift of Dr. Len Post and Dr. Jerry Shen (Biomarin Inc.). PARP inhibitor dose–response survival experiments were carried out in a 6-well plate format, using a 10- to 14-day exposure to PARP inhibitor as previously described (39).
Disclosure of Potential Conflicts of Interest

J. Watkins, A. Grigoriadis, and A.N.J. Tutt have filed a patent application on SCINS method. A. Ashworth may benefit financially from the development of PARP inhibitors through patents held jointly with AstraZeneca through the ICR’s “rewards to inventors” scheme. A.N.J. Tutt has ownership interest (including patents) through The Institute of Cancer Research and King’s College London and has been a consultant/advisory board member for Biomarin and Eisai. No potential conflicts of interest were disclosed by the other authors.

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Other (performed experiments to assess HR level after HORMAD1 expression): P. Vanoli

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