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Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer

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BACKGROUND: Defects in *BRCA1*, *BRCA2*, and other members of the homologous recombination pathway have potential therapeutic relevance when used to support agents that introduce or exploit double-stranded DNA breaks. This study examines the association between homologous recombination defects and genomic patterns of loss of heterozygosity (LOH).

METHODS: Ovarian tumours from two independent data sets were characterised for defects in *BRCA1*, *BRCA2*, and *RAD51C*, and LOH profiles were generated. Publically available data were downloaded for a third independent data set. The same analyses were performed on 57 cancer cell lines.

RESULTS: Loss of heterozygosity regions of intermediate size were observed more frequently in tumours with defective *BRCA1* or *BRCA2* ($P = 10^{-11}$). The homologous recombination deficiency (HRD) score was defined as the number of these regions observed in a tumour sample. The association between HRD score and BRCA deficiency was validated in two independent ovarian cancer data sets ($P = 10^{-5}$ and 10^{-29}), and identified breast and pancreatic cell lines with BRCA defects.

CONCLUSION: The HRD score appears capable of detecting homologous recombination defects regardless of aetiology or mechanism. This score could facilitate the use of PARP inhibitors and platinum in breast, ovarian, and other cancers.

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Hereditary and somatic defects in genes that support homologous recombination (HR) have been implicated in predisposition to a variety of cancers, particularly those affecting breast and ovarian epithelium. The *BRCA1* and *BRCA2* genes are integral members of the HR pathway, are required for functional HR and when disrupted are associated with predisposition to a variety of human cancers (Venkitaraman, 2002). *BRCA1* or *BRCA2* mutations and other HR defects, have potential therapeutic relevance when used to support agents that introduce or exploit double-stranded DNA breaks. For example, better outcomes have been observed in patients with *BRCA1* or *BRCA2* mutations following treatment with DNA-damaging agents such as platinum salts (Tan *et al*, 2008; Hennessy *et al*, 2010), or targeted agents such as PARP inhibitors (PARPi) (Bryant *et al*, 2005; Farmer *et al*, 2005).

Cells deficient for *BRCA1* or *BRCA2* are highly sensitive to *PARP1* inhibitors (Bryant *et al*, 2005; Farmer *et al*, 2005), which are currently in use in clinical trials for cancer patients with *BRCA1* and *BRCA2* germline mutations (Gelmon *et al*, 2011; O'Shaughnessy *et al*, 2011). Recent studies have suggested that HR deficiency in epithelial ovarian cancer (EOC) is not solely due to germline *BRCA1* and *BRCA2* mutations (Hennessy *et al*, 2010; The Cancer Genome Atlas Network (TCGA), 2011; Byler Dann *et al*, 2012). The Cancer Genome Atlas Research Network (2011) reported a defect in at least one HR pathway gene in approximately half of the ~500 EOC in the data set.

Although *BRCA1* and *BRCA2* are thought of primarily as breast and EOC susceptibility genes, mutations in these genes have been reported in a number of other cancers (Friedenson, 2005). Epigenetic silencing of *BRCA1* has also been reported in non-small-cell lung cancer (Marsit *et al*, 2004), cervical cancer (Narayan *et al*, 2003), and EOC (Wilcox *et al*, 2005). Several other HR genes have also been reported to be mutated in other human cancers (Cerbinskaite *et al*, 2011). In addition to the complexity of types of alterations resulting in HR deficiency, each cancer type would appear to have a unique spectrum of HR defects (Cerbinskaite *et al*, 2011).

Defects in the HR pathway might result in specific structural changes in DNA. Previously, chromosomal copy number (CN)

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changes have been reported to be associated with *BRCA1* and *BRCA2* mutations (Stefansson *et al*, 2009; Holstege *et al*, 2010). This study examines the association between genomic patterns of loss of heterozygosity (LOH) and HR deficiency. Loss of heterozygosity was examined in preference to CN because LOH results in the irreversible loss of one of the parental alleles. In contrast, CN gains are not necessarily permanent. Therefore, we hypothesised that, if HR deficiency leaves a footprint of genomic alterations, LOH may provide a more stable record of those changes compared with CN variants. In this study we analysed patterns of genome-wide LOH in three different EOC data sets extensively characterised for *BRCA1* and *BRCA2* defects. An LOH-based score was developed, which is strongly associated with functional defects in *BRCA1* and *BRCA2*. This score also strongly correlates with promoter methylation of *RAD51C*, a gene implicated in the HR pathway.

MATERIALS AND METHODS

Additional information can be found in the Supplementary Methods.

Ovarian tumour samples

Three independent human EOC cohorts were used. 1 : 152 unselected EOC samples from the Gynaecology Cancer Banks at MD Anderson Cancer Centre (MDACC) and the University of California San Francisco (UCSF). Magee-Womens Hospital of UPMC (Pittsburgh, PA, USA) provided 2 : 53 late-stage serous ovarian tumours and 3: 435 serous EOC samples for which complete information was available from The Cancer Genome Atlas Network web site (2011; http://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm?diseaseType=OV). All cohorts were obtained under Institutional Review Board-approved protocols. Patient and tumour characteristics are shown in Supplementary Table S1. Varying numbers of samples were utilised in the assays described (Supplementary Table S2).

Cell lines

Fifty-seven cancer cell lines were analysed (21 ovarian, 32 breast, 3 colon, 1 pancreatic) (see Supplementary Table S3). Two breast cancer cell lines were obtained from DSMZ (Braunschweig, Germany). The colon, pancreatic and remaining breast cancer cell lines were obtained from ATCC (Manassas, VA, USA). Twenty-one EOC cell lines were provided by the Gynaecology Cancer Bank at MD Anderson Cancer Centre (MDACC).

Cell lines obtained from MD Anderson Cancer Centre were validated by STR DNA fingerprinting using the AmpF_STR Identifiler kit according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA; cat 4322288). The STR profiles were compared with known ATCC fingerprints (ATCC.org), and to the Cell Line Integrated Molecular Authentication database (CLIMA) version 0.1.200808 (http://bioinformatics.istge.it/clima/) (Nucleic Acids Research 37:D925-D932 PMCID: PMC2686526). The STR profiles matched known DNA fingerprints or were unique.

Cell lines obtained from DSMZ and ATCC were authenticated using STR DNA typing. Additional information can be found at the DSMZ website (http://old.dsmz.de/human_and_animal_cell_lines/main.php?content_id=86) and in Durkin and Reid (1998).

Extraction of genomic DNA and total RNA from frozen tumours and cell lines

Ten micron frozen sections were cut and macrodissected. The tissue was homogenised (TissueRuptor, Qiagen, Valencia, CA, USA) after addition of QIAzol lysis reagent, following by RNA isolation using a Qiagen miRNAeasy Mini Kit per the manufacturers protocol. A QIAamp DNA Mini Kit (Qiagen) was used to isolate DNA as per the manufacturer's protocol with an overnight lysis incubation at 56 °C and RNase A treatment.

BRCA1 and *BRCA2* sequencing

BRCA1 and *BRCA2* sequencing was performed as previously described (Hennessy *et al*, 2010). Mutations identified were only included in the analyses if classified as deleterious or suspected deleterious based on previously described criteria (Beaudet and Tsui, 1993).

Promoter methylation qPCR assays

The Methyl-Profiler DNA Methylation PCR Array System (SABiosciences, Valencia, CA, USA) was used to quantify methylation levels following the manufacturers recommended protocol. Assay details are provided in Supplementary Table S4.

Methylation microarrays

Methylation data from TCGA were generated using the Infinium HumanMethylation27 microarray (Illumina, San Diego, CA, USA). Data were downloaded from the TCGA Network website and analysed as described in the Supplemental Methods.

BRCA1 and cell cycle progression signature expression assays

Assays were performed using TaqMan assays (Applied Biosystems) on a Fluidigm (San Francisco, CA, USA) BioMark instrument.

Identification of samples with loss of BRCA1 expression The method used is described in the Supplemental Methods. Assay details are provided in Supplementary Table S5.

Affymetrix 500 K genechip arrays

The Affymetrix (Santa Clara, CA, USA) GeneChip Mapping *Nsp*I or *Sty*I microarrays were run according to the manufacturer's protocol.

Copy number and LOH analysis of SNP microarray data

Copy number and LOH analysis was performed using an algorithm described in the Supplemental Methods. In addition to reconstructing CN and LOH profile, the algorithm estimates contamination with normal DNA.

Statistical analysis

The molecular assays used in the study are unreliable at high levels of contamination of the tumour samples with normal tissue, therefore samples with estimated contamination above 65% were excluded from statistical analyses (the rationale for this cutoff is explained in the Supplemental Results and in Supplemental Figures S7, S8, and S9). *P*-values in this paper were calculated using Kolmogorov–Smirnov test unless otherwise specified. A comparison of the *P*-values obtained using the Kolmogorov–Smirnov and Fisher tests is presented in the Supplemental Methods.

RESULTS

Homologous recombination-deficient tumours

A tumour sample was considered HR deficient if it had a germline or somatic mutation in *BRCA1* or *BRCA2*, methylation, or low

mRNA expression of *BRCA1* and also demonstrated LOH for *BRCA1* or *BRCA2*. Methylation and expression analysis of *BRCA2* did not identify any samples harbouring these defects. Thirty-one of 152 samples from the first cohort were carriers of mutations in *BRCA1* and/or *BRCA2*, along with 14/53 from the second cohort and 83/435 from the third cohort (two of which were excluded from further analysis, see below). Mutations are summarised in Supplementary Table S6.

The degree of methylation was measured for promoter CpG islands of both *BRCA1* and *BRCA2*. Methylation in multiple samples was observed for *BRCA1*, but not *BRCA2*. Eleven of 126 samples from the first cohort (Supplementary Figure S1), 3 of 34 from the second cohort and 64 of 435 from the third cohort were defined as HR deficient due to high levels of *BRCA1* promoter methylation. With the exception of one sample from the third cohort, none of the samples with a methylated *BRCA1* promoter had concordant *BRCA1* or *BRCA2* mutations.

Mechanisms other than promoter methylation could result in low mRNA expression of *BRCA1* or *BRCA2* that might also lead to HR deficiency. *BRCA1* and *BRCA2* expression levels were measured for 137 samples from the first cohort and 53 samples from the second cohort. Expression of *BRCA1* in 20 samples was abnormally low. Only five samples with abnormally low expression of *BRCA1* were not flagged as HR deficient because of *BRCA1* promoter methylation (Supplementary Figure S2). No abnormally low expression was observed for *BRCA2*.

In this analysis, samples were only classified as BRCA deficient if *BRCA1* or *BRCA2* was contained within a region of LOH and carried a mutation, was methylated, or was expressed at low levels. Two *BRCA2*-mutant samples that did not have LOH were not considered HR deficient in our analysis. In total, in the three data sets analysed ($n = 640$) there were 211 samples with *BRCA1* or *BRCA2* mutations, methylation, or low expression. In all but two of these cases, both with *BRCA2* mutations, there was homozygosity at the affected gene due to LOH.

Distribution of lengths of LOH regions

SNP analysis was used to determine the distribution of lengths of LOH regions throughout the genome. The lengths of LOH regions adjusted to the length of chromosome arm on which the LOH regions were observed are shown in Figure 1. Three distinct features were observed in this distribution. First, there are many short LOH regions (<15 Mb). Second, there is a long flat tail of LOH regions up to the length of a single chromosome arm with few LOH regions covering more than one chromosome arm but less than the whole chromosome. Finally, there is a high peak corresponding to LOH over the whole chromosome.

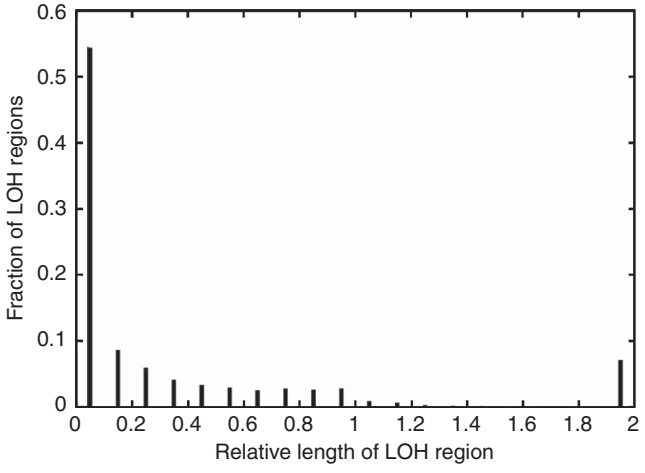

Figure 1 Fraction of lengths of LOH regions *vs* length of these regions adjusted to the length of chromosome arm. Chromosomes 13, 14, 15, and 22 were excluded because SNPs are not available for the p arms of these chromosomes. The largest adjusted value of 2 corresponds to LOH over the entire chromosome.

Correlation between samples with HR deficiency and LOH

At high levels of contamination of a tumour sample with normal tissue the assays used in the study might be unreliable. Although LOH reconstruction from SNP microarray data appear to be robust up to 85% contamination and mutation detection results up to 90% contamination, methylation and RNA expression assays are more sensitive to contamination and become unreliable above 65% of contamination (Supplementary Results). Therefore, samples with contamination above 65% have been excluded from the analysis: 20 samples from the first cohort (two of them with *BRCA1* mutations), 10 samples from the second cohort (one of them with a *BRCA1* mutation), and one sample from the third cohort.

The first cohort of samples was used as the 'discovery' cohort. LOH regions on chromosome 17 were excluded from the analysis, because LOH was observed over this chromosome in almost all samples. We checked for correlation between HR deficiency and the number of short LOH regions (<15 Mb), the number of long LOH regions (>15 Mb but less than the whole chromosome), and the number of LOH regions covering whole chromosomes. The value of 15 Mb was selected somewhat arbitrarily, but further analysis showed that the exact value of this cut-off does not have significant impact on the results (Supplementary Figure S4). There was no significant correlation between the number of short LOH regions and HR deficiency. The number of LOH regions covering the whole chromosome was significantly greater in tumours with intact *BRCA1* or *BRCA2* ($P = 4 \times 10^{-5}$) (Supplementary Figure S3a). The number of long LOH regions (>15 Mb but less than the whole chromosome) was significantly higher in tumours with deficient *BRCA1* or *BRCA2* ($P = 10^{-11}$) (Figure 2A). From these samples, we determined a homologous recombination deficiency (HRD) score defined as the number of LOH regions >15 Mb, but less than a whole chromosome in length, within a tumour genome.

The second and third cohorts were used to validate the results obtained for the first cohort. Homologous recombination deficiency was not correlated with the number of LOH regions covering whole chromosomes in the second cohort, possibly due to low sample number, but this observation was confirmed in the third cohort ($P = 3 \times 10^{-11}$) (Supplementary Figure S3b). Highly significant correlation was observed between HRD score and HR deficiency for both cohorts ($P = 10^{-5}$ and $P = 10^{-29}$, respectively) with HRD score being distinctly reduced among ovarian tumours with intact *BRCA1* and *BRCA2* (Figures 2B and C).

Alterations in *RAD51C* and other HR pathway genes

Published data suggest that *BRCA1* and *BRCA2* are the primary genes responsible for HR deficiency in EOC (TCGA, 2011). However, many other genes may also be important, such as *RAD51C* and *RAD51D* both of which have been recently been implicated as predisposition genes for EOC (Meindl *et al*, 2010; Loveday *et al*, 2011). In the TCGA data set there were no *RAD51C* or *RAD51D* mutations reported; however, 11/435 samples had methylation of the *RAD51C* promoter. In addition, the degree of methylation was measured for promoter CpG islands of eight genes involved in the HR pathway in addition to *BRCA1* and *BRCA2* (Supplementary Table S4) in the first cohort. Only *RAD51C* had high levels of promoter methylation in 3 of 89 samples. All samples positive for *RAD51C* methylation from both cohorts were homozygous at the *RAD51C* locus because of LOH. To test whether the HRD score is elevated in samples with *RAD51C* promoter methylation samples from both cohorts were compared

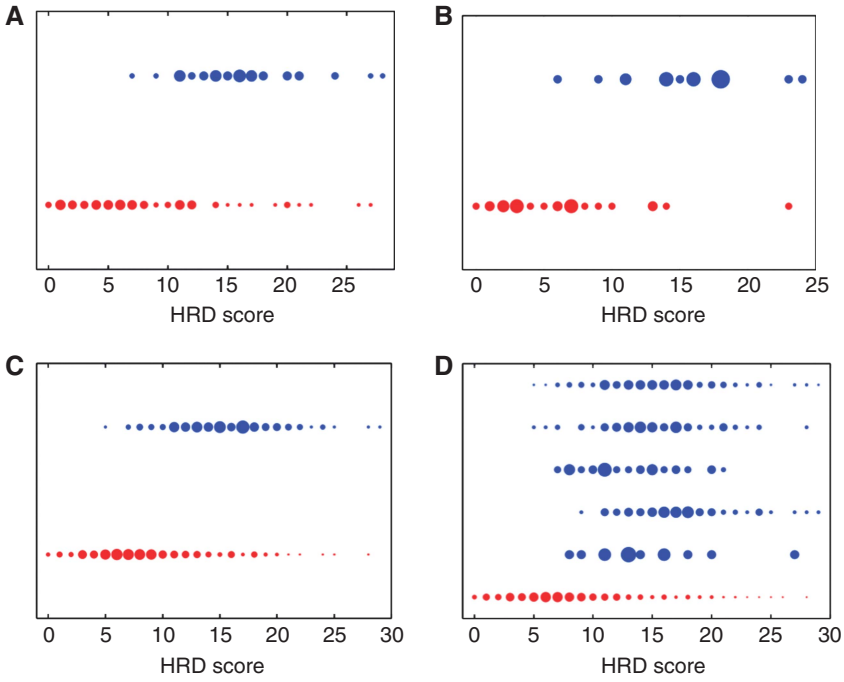

Figure 2 Homologous recombination deficiency score in tumour samples. The number of long LOH regions is shown on the x axis. Blue circles: *BRCA1*- or *BRCA2*-deficient samples. Red circles: *BRCA1*- and *BRCA2*-intact samples. The combined area under the blue and red circles is the same. This normalisation is used only to facilitate visualisation of the circles. The size of each individual circle is proportional to the number of samples with the corresponding number of LOH regions. (**A**) Homologous recombination deficiency score for the first cohort (44 of 132 samples were *BRCA1* or *BRCA2* deficient). (**B**) Homologous recombination deficiency score for the second cohort (18 of 43 samples were *BRCA1* or *BRCA2* deficient). (**C**) Homologous recombination deficiency score for the third cohort (146 of 434 samples were *BRCA1* or *BRCA2* deficient). (**D**) Homologous recombination deficiency score for the combined data from all three cohorts. Row A: 221 samples with either *BRCA1*, or *BRCA2*, or *RAD51C* deficient genes; B: 80 *BRCA1* mutants (a *BRCA1* mutant with a *BRCA2* mutation, a *BRCA1* mutant with a methylated *BRCA1* promoter, and a *BRCA1* mutant with a methylated *RAD51C* promoter were excluded from this plot); C: 43 *BRCA2* mutants (a *BRCA2* mutant with a *BRCA1* mutation was excluded from this plot); D: 82 samples with low expression or methylation of *BRCA1* (a sample with a methylated *BRCA1* promoter and a *BRCA1* mutation was excluded from this plot); E: 13 samples with methylation of *RAD51C* (a sample with a methylated *RAD51C* promoter and a *BRCA1* mutation was excluded from this plot). Red circles: 388 samples with *BRCA1*, *BRCA2*, and *RAD51C* intact genes.

with BRCA intact samples without *RAD51C* methylation. Consistent with our observations for *BRCA1* and *BRCA2* genes, HRD score was significantly higher ($P = 0.0003$) among samples with *RAD51C* methylation.

It has been reported that *PTEN*-deficient tumour cells are sensitive to PARPi, and suggested that deficiency in *PTEN* results in HR pathway defects (Mendes-Pereira *et al*, 2009). Copy number analysis revealed 2/132 samples from the first data set (HRD scores = 11 and 21) and 0/43 samples from the second data set had homozygous deletions of *PTEN*. Both of the *PTEN*-deficient samples also had defects in either *BRCA1* or *BRCA2*. In the third data set 11/435 samples had homozygous deletions of *PTEN*, and their HRD scores ranged from 9–22. An additional 3/435 samples had likely deleterious mutations (nonsense, frameshift, or splice-site mutations) in *PTEN* with homozygosity at the *PTEN* locus because of LOH (HRD scores = 8, 16, 24). Five of these samples had defects in *BRCA1*, *BRCA2*, or *RAD51C*. The remaining nine samples had a significantly elevated ($P = 0.004$) average HRD score of 14.5 (range 8–24) consistent with *PTEN* having a role in homologous recombination.

In the third cohort presumed deleterious mutations and methylation of HR pathway genes have been reported (TCGA, 2011). The mutations were examined and the analysis was limited to defects with a high likelihood of being deleterious (for example, nonsense and frameshift mutations), resulting in a total of eight deleterious mutations in six genes (*ATM*, *ATR*, *FANCA*, *FANCD2*, *FANCM*, and *PALB2*) not discussed above. An additional five samples had methylation of HR pathway genes. Loss of heterozygosity was detected in only 1 of the 13 samples (a *FANCM* nonsense mutation). As deactivation of both alleles is needed to lose function of a tumour suppressor, 12 of these 13 samples are expected to have intact HR. Not surprisingly, the HRD score was not elevated in the majority of these samples (data not shown).

Analysis of combined data

Correlation between the HRD score and HR deficiency (defined as deficiency of *BRCA1*, *BRCA2*, or *RAD51C*) for all three cohorts is presented in Figure 2D. A highly significant association is seen ($P = 2 \times 10^{-48}$) (Table 1).

An important question is whether the distribution of HRD scores is the same for HR deficiency because of different genomic loci. To answer this, the distributions of HRD scores for *BRCA1*-, *BRCA2*-, and *RAD51C*-deficient tumours were analysed separately (Supplementary Figure S6). A significant difference was observed ($P = 6 \times 10^{-5}$) with *BRCA1*-deficient samples a having higher average HRD score (16.2; s.d. = 4.3) than *BRCA2*-deficient samples (13.0; s.d. = 3.9). The differences in HRD scores between either *BRCA1* or *BRCA2* and *RAD51C* (14.5; s.d. = 5.1) were not significant.

To determine whether mutations in *BRCA1* and *BRCA2* were germline or somatic, normal tissue was analysed when available. A subset of normal samples from the first and second cohorts, and all samples from the third cohort were available. There was no significant difference in the distributions of HRD scores for either *BRCA1* or *BRCA2* deficiency between germline and somatic mutations, or between mutations and either methylation or low expression (Supplementary Figure S5).

Table 1 Average of HRD score for *BRCA1* and *BRCA2* deficient and intact tumours and corresponding *P*-values

	HR deficient (*BRCA1* and *BRCA2*)	HR intact (*BRCA1* and *BRCA2*)	HR deficient (*BRCA1*, *BRCA2*, and *RAD51C*)	HR intact (*BRCA1*, *BRCA2*, and *RAD51C*)
First cohort	16.1 (s.d. = 4.5)	7.9 (s.d. = 6.1)	16.5 (s.d. = 4.7)	7.6 (s.d. = 5.6)
	$P = 10^{-11}$		$P = 10^{-12}$	
Second cohort	15.5 (s.d. = 4.5)	6.4 (s.d. = 5.2)	15.5 (s.d. = 4.5)	6.4 (s.d. = 5.2)
	$P = 10^{-5}$		$P = 10^{-5}$	
Third cohort	15.3 (s.d. = 4.3)	8.8 (s.d. = 5.0)	15.1 (s.d. = 4.3)	8.6 (s.d. = 5.0)
	$P = 10^{-29}$		$P = 3 \times 10^{-32}$	
Combined data for three cohorts	15.5 (s.d. = 4.4)	8.4 (s.d. = 5.3)	15.4 (s.d. = 4.4)	8.2 (s.d. = 5.2)
	$P = 7 \times 10^{-45}$		$P = 2 \times 10^{-48}$	
Cancer cell lines	19.9 (s.d. = 5.0)	8.0 (s.d. = 5.5)	19.9 (s.d. = 5.0)	8.0 (s.d. = 5.5)
	$P = 0.0001$		$P = 0.0001$	

Abbreviations: HR = homologous recombination; HRD = homologous recombination deficiency.

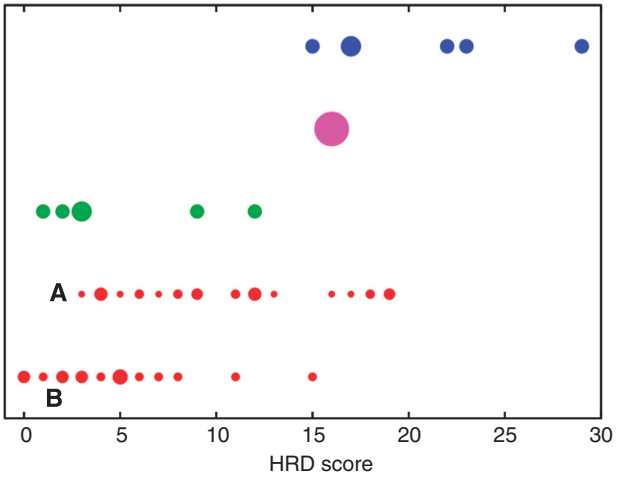

Figure 3 Comparison of HRD scores in cancer cell lines. Red circles: cell lines with intact *BRCA1* or *BRCA2*. (**A**) 28 non-ovarian cell lines; (**B**) 16 ovarian cell lines. Green circles: six carriers of heterozygous mutations in either *BRCA1* or *BRCA2*. Violet circles: one carrier of a homozygous mutation with reversion in *BRCA1*. Blue circles: six carriers of homozygous mutations in either *BRCA1* or *BRCA2* or with methylated *BRCA1*. The combined area under the green, red, blue and violet circles is the same. The size of each individual circle is proportional to the number of samples with the corresponding number of LOH regions.

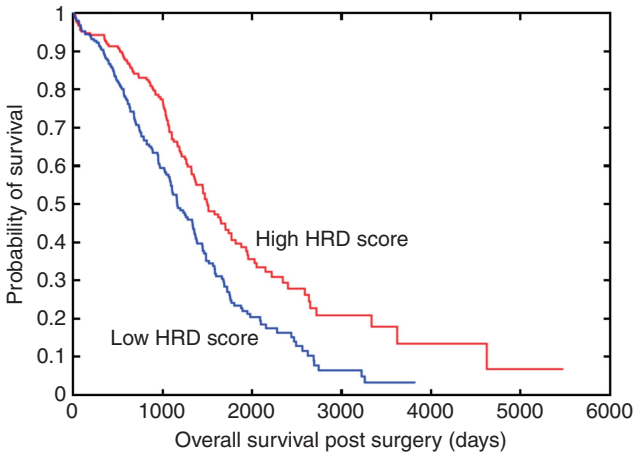

Figure 4 Kaplan–Meier plot of OS post surgery for HRD score split at it's median. These data were generated using 507 samples from the TCGA data set for which copy number data and survival information were available. Median OS for samples with high and low HRD score were 1499 (95% CI = (1355–1769)) and 1163 (95% CI = (1081–1354)) days, respectively. The *P*-value was calculated using Cox model.

Homologous recombination deficiency score in *BRCA1*- and *BRCA2*-deficient cell lines

Unselected breast ($n = 32$) and ovarian ($n = 21$) cell lines were obtained from multiple sources and screened for mutations in *BRCA1* or *BRCA2*. In addition three colon and one pancreatic cell line from NCI60 were analysed. Of these 57 cell lines, six either carried homozygous deleterious mutations or had methylation of the *BRCA1* promoter and LOH at *BRCA1*, one had a homozygous mutation with an apparent functional reversion, and six carried heterozygous mutations (mutations are described in Supplementary Table S7). Figure 3 shows the distributions of HRD scores for these three groups of mutants, as well as for samples that do not carry BRCA mutations. The distributions of HRD scores among ovarian tumours and cancer cell lines without BRCA defects were not significantly different. The distribution of HRD scores among cancer cell lines with heterozygous mutations is similar to cancer cell lines without BRCA defects, presumably because cells become HR deficient only when both copies of *BRCA1* or *BRCA2* are non-functional. For cancer cell lines with functional loss of both copies of either *BRCA1* or *BRCA2*, higher HRD scores are observed, similar to HRD scores observed for ovarian tumours with *BRCA1*-, *BRCA2*-, or *RAD51C*-deficient genes. The HRD score is also high for the cancer cell line with reversion of a *BRCA1* mutation. The difference of the distribution of HRD scores in either wild-type or heterozygous mutant cell lines, and the distribution of HRD scores in cell lines with either homozygous mutations (with or without reversion) or methylation of the *BRCA1* promoter is highly significant ($P = 0.0001$). Importantly, there is significant correlation between HRD score and *BRCA1* and *BRCA2* deficiency even after excluding EOC cell lines from the data set ($P = 0.01$), suggesting that association of HRD score with HR deficiency is not restricted to EOC.

Correlation between HR deficiency and overall survival (OS) and progression free survival (PFS)

Correlation was not detected between HRD score and either OS or PFS for the first two cohorts, possibly because of cohort size. Significant correlation was observed between PFS ($P = 0.03$) and OS ($P = 6 \times 10^{-5}$) for the third cohort with improved survival for patients with higher HRD scores (Figure 4).

DISCUSSION

This study reports the development of a DNA-based HRD score based on genome-wide LOH analysis of ovarian tumours combined with comprehensive profiling of *BRCA1* and *BRCA2* defects. The HRD score is strongly associated with the integrity of homologous recombination, as measured by *BRCA1*, *BRCA2*, and *RAD51C* defects. The correlation of HRD score and HR deficiency was validated in two independent EOC data sets, as well as breast and pancreatic cancer cell lines.

The initial hypothesis was that regions of LOH of different length might appear in the cancer genome through different pathways, thus tumours with HR deficiency might manifest a specific pattern of LOH. In this study, we identified an intermediate class of LOH sizes >15 Mb but less than a whole chromosome that was highly positively correlated with defective HR genes suggesting that most if not all, of this type of LOH class exists, because it incorporates double-stranded DNA breaks as part of its genesis and requires repair by HR. In contrast, LOH at the whole chromosome level is significantly less frequent in HR-deficient tumours. One possible explanation is that LOH at the whole chromosome level frequently originates through an alternative mechanism that does not involve double-stranded DNA breaks.

In addition to *BRCA1* and *BRCA2* defects, *RAD51C* promoter methylation was observed in ovarian tumours. High HRD score was significantly associated with *RAD51C* deficiency in two data sets. Elevated HRD scores were also seen in *PTEN*-deficient tumours, consistent with reports that *PTEN*-defective tumour cell are HR defective (Mendes-Pereira *et al*, 2009). Only one additional HR gene mutation was confirmed in the three data sets, a nonsense mutation in *FANCM* with LOH resulting in loss of the second allele. The HRD score associated with the *FANCM* mutation (TCGA, 2011) is within the range of the normal distribution for samples with elevated HRD score.

Among tumours with apparently intact *BRCA1*, *BRCA2*, and *RAD51C,* a substantial fraction of the samples have an elevated HRD score. Two possible explanations are that there is a substantial rate of defects in other genes in the HR pathway in many of these samples, or that the HRD score is non-specific.

Published studies have demonstrated that secondary reversion mutations that restore *BRCA2* function can arise in *BRCA2* mutant cell lines after exposure to platinum agents (Edwards *et al*, 2008; Sakai *et al*, 2008, 2009). Norquist *et al* (2011) observed similar findings *in vivo*, with $\sim$28% of recurrent tumours having a secondary mutation that restored *BRCA1* or *BRCA2* function. Reversion mutations were observed primarily in individuals with prior exposure to platinum agents and were predictive of resistance to platinum. As the HRD score results from cumulative defects occurring in the genome of the tumour, it should not be sensitive to reversion mutations. Post-treatment samples were not available from the tumours used in this study, however, the limited data obtained from cell lines with reversion mutations is consistent with this hypothesis. It should be noted that the insensitivity of the HRD score to reversion mutations would affect few tumours in the neoadjuvant or adjuvant setting (Norquist *et al*, 2011).

Recently, Birkbak *et al* (2012) have reported a telomeric allelic imbalance score, which predicted pathological complete response to cisplatin treatment in triple negative breast cancer patients. Although the score itself is very different from the score we have identified, which is based solely on LOH, future plans include investigating how well correlated the HRD and TAI scores are with one another. In addition, we would like to determine whether the TAI score is associated with *BRCA1*, *BRCA2*, and *RAD51C* defects in our cohorts similar to the HRD score.

Current studies are focused on evaluating the ability of the HRD score to identify BRCA-deficient tumours in breast and pancreatic cancer data sets. If the HRD score is validated as a surrogate marker for HR deficiency, regardless of mechanism, it could be used as a quick and reliable assay for tumour stratification. In addition, studies are underway to investigate the ability of this score to predict response to both platinum agents and PARPi. The current data suggest that a high HRD score is highly correlated with HR deficiency, and that this score could be utilised to identify patients with high likelihood of responding to DNA damaging agents and PARPi. Such a test would have clinical utility in breast and EOC, and could conceivably be used to expand the use of PARPi and platinum salts to other cancers.

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Conflict of interest

VA, KMT, JP, JC, TVT, DW, DI, SJ, LF, AG, and JSL are employees of Myriad Genetics, Inc. The remaining authors declare no conflict of interest.

Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc)

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