Research

Aberrant *PRDM9* expression impacts the pan-cancer genomic landscape

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The binding of *PRDM9* to chromatin is a key step in the induction of DNA double-strand breaks associated with meiotic recombination hotspots; it is normally expressed solely in germ cells. We interrogated 1879 cancer samples in 39 different cancer types and found that *PRDM9* is unexpectedly expressed in 20% of these tumors even after stringent gene homology correction. The expression levels of *PRDM9* in tumors are significantly higher than those found in healthy neighboring tissues and in healthy nongerm tissue databases. Recurrently mutated regions located within 5 Mb of the *PRDM9* loci, as well as differentially expressed genes in meiotic pathways, correlate with *PRDM9* expression. In samples with aberrant *PRDM9* expression, structural variant breakpoints frequently neighbor the DNA motif recognized by *PRDM9*, and there is an enrichment of structural variants at sites of known meiotic *PRDM9* activity. This study is the first to provide evidence of an association between aberrant expression of the meiosis-specific gene *PRDM9* with genomic instability in cancer.

[Supplemental material is available for this article.]

Proper chromatid segregation and genome stability are dependent on recombination between homologous chromosomes during meiosis (Baker et al. 1976; Purandare and Patel 1997; Baudat and de Massy 2007; Alves et al. 2017). Meiotic recombination events do not occur across most of the human genome but cluster in 1- to 2-kb-wide hotspots (Pratto et al. 2014) whose locations are associated with activity of the protein PR/SET domain 9 (*PRDM9*) (Baudat et al. 2010; Myers et al. 2010; Parvanov et al. 2010). The functional domains of *PRDM9* include a Krüppel-associated box (KRAB)-related domain, which facilitates protein-protein interactions; a PR/SET domain, which provides the protein with H3K4me3 and H3K36me3 activity (Blazer et al. 2016; Powers et al. 2016); and a DNA-binding zinc finger (ZnF) array domain. The SET domain places epigenetic marks at sites bearing specific motifs recognized by the ZnF domain, leading to the recruitment of the DNA double-strand break (DSB) and meiotic recombination machinery (Smagulova et al. 2011; Brick et al. 2012; Pratto et al. 2014). *PRDM9* expression and associated epigenetic marks disappear during the pachytene stage of meiosis, leading to the inference that *PRDM9*'s biological function is exclusive to gametogenic tissue (Sun et al. 2015). By directing sites of DSBs, *PRDM9* contributes to the tight regulation of meiotic recombination processes, which ensures the stability of homologous chromosomes and proper chromosomal disjunction (Baker et al. 1976; Purandare and Patel 1997; Baudat and de Massy 2007; Alves et al. 2017). While *PRDM9* expression has previously been observed in cancer cell lines, as well as in five ovarian carcinomas and a lung adenocarcinoma (Feichtinger et al. 2012), a broad characterization of its expression and implications

on the transcriptomic and genomic landscape across cancer types is still lacking.

In healthy nongerm cells, genome integrity is dependent on recombination: The homologous recombination repair pathway allows for the conservative repair of DSBs in DNA caused by endogenous or exogenous damage (Andersen and Sekelsky 2010; Symington et al. 2014; Lisby and Rothstein 2016). When DSBs are not immediately repaired, they can result in structural aberrations such as translocations, inversions, and deletions through nonspecific repair mechanisms (Jackson 2002). A high rate of somatic structural variants (SVs) is characteristic of genomic instability, one of the hallmarks of cancer (Hanahan and Weinberg 2011). DSB repair (DSBR) deficiency underlies a mutational signature that has been associated with germline and somatic loss-of-function (LOF) mutations (Alexandrov et al. 2013) in genes involved in the homologous recombination repair pathway (Moynahan and Jasin 2010; Krejci et al. 2012; Lord and Ashworth 2016). However, some cancers exhibit the mutational signatures of DSBR deficiencies despite lacking detectable LOF mutations affecting known regulators in the homologous recombination repair pathway (Connor et al. 2017), indicating the importance of understanding underlying causes of genomic instability.

Here, we describe aberrant expression of *PRDM9* among human tumors in vivo in 32 different cancer types (from $n = 1879$ patients) from the Pan-Cancer Analysis of Whole Genomes Project (PCAWG) and The Cancer Genome Atlas (TCGA) and assess the impact of this aberrant expression on the genomic and transcriptomic landscape among tumors.

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Results

Aberrant *PRDM9* expression is common in human cancers

We first evaluated *PRDM9* expression in two large cancer cohorts: PCAWG and TCGA (data described in [Supplemental Fig. S1](#)) (The Cancer Genome Atlas Research Network et al. 2013). Overall, 365 tumor samples expressed *PRDM9* across 32 cancer types (Fig. 1A). Cancers exhibiting the highest expression include head and neck squamous cell carcinoma and bladder urothelial carcinoma, with median expression levels of 153.84 and 95.41 fragments per kilobase of transcript per million mapped reads-upper quartile (FPKM-UQ), respectively. We compared *PRDM9* expression levels to other expressed genes within each sample by partitioning gene expression levels into percentiles. *PRDM9* expression ranged from the second to the 73rd percentile of gene expression values with a mean of the 10th percentile across all samples. Liver and ovarian cancers in particular had a high proportion of tumors exhibiting *PRDM9* expression with, respectively, 46% and 44% of tumors expressing *PRDM9* above a threshold of 10 FPKM-UQ ([Supplemental Fig. S3](#)). Among most tumor sample pairs expressing *PRDM9*, higher expression of *PRDM9* was found compared to the matching normal sample (one-sided Wilcoxon signed-rank test: $P < 2.2 \times 10^{-16}$) (Fig. 1B), suggesting that *PRDM9* expression levels in tumors are higher than those of healthy tissues. To account for any effects possibly originating from tumor infiltration in the surrounding tissue, we next compared expression data in healthy tissues from the Genotype-Tissue Expression (GTEx) project (Melé et al. 2015) to transcriptome data from tumor samples (Fig. 1C). Again, the proportion of samples expressing *PRDM9* was significantly higher among tumor transcriptomes compared with the cohort of non-germ healthy tissues, where nearly no *PRDM9* expression was observed (one-sided two-sample test for equality of proportions: $P < 2.2 \times 10^{-16}$) (Fig. 1C). Taken together, these analyses confirm that *PRDM9* becomes transcriptionally active in many cancers, a feature that is characteristic to cancer in nongerm cells. In the following sections, we focus on the PCAWG cohorts for further analyses to evaluate associations between *PRDM9* expression and the genomic and transcriptomic landscape, due to the availability of whole-genome sequencing data for these donors.

Aberrant *PRDM9* expression is associated with differentially expressed genes

To identify transcripts whose expression was associated with aberrant *PRDM9* expression, we performed a differential gene expression (DGE) analysis (Love et al. 2014), comparing tumors expressing *PRDM9* to those without any detectable *PRDM9* transcripts (Fig. 2A). Overall, 3114 genes were differentially expressed ($FDR < 1\%$) in tumors expressing *PRDM9*, including 22 cancer driver genes identified in the IntOGen resource (Gonzalez-Perez et al. 2013). Out of 43 genes differentially expressed in cell lines with *PRDM9* transfection (Altemose et al. 2017), nine were also differentially expressed in our analysis ([Supplemental Data 1.1](#)). Among the differentially expressed genes, we identify 13 genes with functions associated to meiotic processes, including the up-regulation of *REC8*, a gene coding for a meiosis-specific member of the cohesin complex (Watanabe and Nurse 1999), and of *MIAP*, whose protein likely contributes to the coordination of meiotic processes (Arango et al. 2013). As well, Gene Ontology (GO) biological processes involving cell differentiation, G-protein signaling, and nucleosome assembly were enriched in pan-cancer highly interacting modules of differentially expressed genes asso-

ciated with *PRDM9* ([Supplemental Fig. S4](#)), similar to biological processes enriched in modules of genes with testes-specific expression, which are suggestive of meiotic processes. The overlap in biological processes between modules built from these two different sets of genes suggests common processes between testes and cancers expressing *PRDM9*. Additionally, 13 out of the 241 genes associated with the meiosis biological process were differentially expressed in our analysis ([Supplemental Data 1.1](#)).

When a distinct DGE analysis was conducted separately for each cancer type, the median number of differentially expressed genes associated with *PRDM9* expression across cancer types was 122.5, with the lowest being three (renal cell cancer) and the highest being 808 (liver hepatocellular carcinoma). A total of 245 genes were differentially coexpressed with *PRDM9* in two or more unique cancer types ([Supplemental Fig. S5](#); [Supplemental Data 1.2](#)).

Additionally, we performed DGE analyses to determine whether there was a sex-biased expression of genes correlated with *PRDM9* expression. We found no significant association between the sex of patients and *PRDM9* expression (χ^2 test: $P = 0.90$). However, when we performed similar DGE analyses using sex as a cofactor (again including cancer types; see Methods), 1178 genes were differentially expressed ($FDR < 1\%$) ([Supplemental Data 1.3](#)), including 864 that were not differentially expressed in the previous analysis that did not include sex as a cofactor. Some of these genes have expression specific to testis, such as *TEX41*, *TSKS*, *BRDT*, and *TSGA10IP*, and others specific to meiotic processes, such as *DMC1* and *EME1*. In [Supplemental Data 1.4](#), we show 507 genes that were differentially expressed in at least two different cancer types when each cancer type was analyzed separately and sex was still included as a cofactor. These results indicate that, in cancer, *PRDM9* expression was associated with the sex-biased expression of other loci.

Recurrently mutated regions are associated with *PRDM9* expression in tumors

We next investigated whether somatic mutations were associated with *PRDM9* expression. Because identical recurrent somatic variants are extremely rare, we identified regions where SNVs cluster across all tumor samples: Such regions had at least two SNVs located no more than 100 bp apart. We identified 1,507,106 such regions and concentrated on the 5548 that exhibited a mutation in at least 5% of the samples (Lamaze et al. 2017). We tested for associations between *PRDM9* expression levels and each of the 5548 recurrently mutated regions in order to identify somatic alterations that may contribute to the aberrant expression of *PRDM9* (Methods). Forty-nine recurrently mutated regions were significantly associated with aberrant *PRDM9* expression (Fig. 2B; [Supplemental Data 2](#)). Of these 49 regions, 13 were located on Chromosome 5, where *PRDM9* is also located, and of them, eight were located within 5 Mb of the *PRDM9* locus. Several genes proximal to these eight recurrently mutated regions have testes-specific expression in data from the GTEx Consortium (Chr 5: 24,349,767–24,353,800, CTD-2074D8.1; Chr 5: 24,852,561–24,856,027, RP11-730N24.1; Chr 5: 28,123,525–28,127,367, RP11-560A7.1; Chr 5: 29,117,954–29,121,731, RP11-42L13.3), similar to *PRDM9* ([Supplemental Data 2](#)).

PRDM9 binding motif locations are significantly associated with somatic structural breakpoints

We investigated the downstream effects of *PRDM9* expression among tumors by testing for associations of *PRDM9* expression

Figure 1. Characterization of aberrant *PRDM9* expression across cancer types. (A) A total of 365 samples expressing *PRDM9* above 10 FPKM-UQ across 39 cancer types from the PCAWG and TCGA data sets ($n=1879$), after a correction to account for the high homology between *PRDM9* and *PRDM7* (Methods; see Supplemental Fig. S2). Black squares represent the median of *PRDM9* expression above a threshold of 10 FPKM-UQ within each cancer type. (B) *PRDM9* expression in 128 tumor samples expressing *PRDM9* above a threshold of 10 FPKM-UQ and their matching normal samples, across cancer types within the TCGA and the PCAWG cohorts ($n=813$ pairs). Data points are colored according to cancer type, and lines connect tumor and normal samples originating from the same patient. Cancer samples have higher *PRDM9* expression than their matching healthy tissues (one-sided Wilcoxon signed-rank test: $P < 2.2 \times 10^{-16}$), a result consistent within each cancer type where at least three sample pairs were expressing *PRDM9* (Fisher's method with one-sided Wilcoxon signed-rank test: $P = 2.28 \times 10^{-20}$) (Supplemental Table S1). (C) Proportion of samples expressing *PRDM9* in the cancer cohorts (PCAWG and TCGA) compared with the proportion of samples expressing *PRDM9* in the GTEx cohort, excluding testes. The proportion of samples expressing *PRDM9* in cancer samples is significantly higher than that of healthy tissues.

Figure 2. Differentially expressed genes and recurrently mutated regions are associated with *PRDM9* expression in cancer. (A) A total of 3114 genes were differentially expressed (in purple) above a threshold of 2 $\log_2$ fold change and a *P*-value adjusted for multiple testing using the Benjamini-Hochberg procedure ($FDR < 0.05$) between groups of cancers partitioned on *PRDM9* expression. Of these genes, 2224 were overexpressed in cancers expressing *PRDM9*, and 890 were underexpressed. Twenty-two known cancer driver genes (Gonzalez-Perez et al. 2013) were differentially expressed in tumors expressing *PRDM9* (in teal), and 13 had functions related to meiosis (in pink). (B) *P*-values from the associations between *PRDM9* expression levels and recurrently mutated regions. The red line shows $P < 0.05$, with a Bonferroni correction for multiple testing for the number of tested regions ($n = 1,507,106$). Forty-nine recurrently mutated regions were significantly associated with aberrant *PRDM9* expression across cancers. Highlighted with pink triangles are loci significantly associated with aberrant expression located on Chromosome 5, where the *PRDM9* locus is located.

with the landscape of somatic SVs. Because the binding of PRDM9 to chromatin during meiosis leads to the recruitment of the DSB machinery, we hypothesized that *PRDM9*-expressing cancers would exhibit an enrichment of SV breakpoints, which may be indicative of DSBs near PRDM9 binding sites. To explore this hypothesis, we investigated whether the DNA motif known to be recognized by PRDM9 (Pratto et al. 2014) was overrepresented in the flanking sequences of somatic SVs (SV breakpoint sequences [SVBSs]) (Supplemental Fig. S6; Li et al. 2017). For samples expressing *PRDM9*, SVBSs were queried using a curated set of sequence motifs recognized by transcription factors (Mathelier et al. 2016), including the motif recognized by the A-variant of PRDM9 (Pratto et al. 2014). The allelic frequency of the A allele is 86% in Europeans and other non-African populations, and is 50% within African populations (Parvanov et al. 2010; Ponting 2011). Non-African individuals comprised the majority of the PCAWG cohort at 86.3% of the samples with expression data. We found that the motif recognized by the PRDM9 A-variant significantly matched the most SVBSs consistently across all cancer types (Fig. 3A). The proportion of SVBSs matching the PRDM9 A-variant binding motif was significantly higher than the proportion of SVBSs matching the ZNF263 binding motif, the second motif most frequently matching SVBSs (Wilcoxon signed-rank test $P = 2.38 \times 10^{-7}$). The proportion of SVBSs significantly matching the PRDM9 motif was also significantly higher in samples expressing *PRDM9* than in samples not expressing *PRDM9* (χ^2 : $P = 2.2 \times 10^{-16}$). Additionally, we performed a discriminative motif capture to identify motifs whose presence discriminated between *PRDM9*-expressing and non-*PRDM9*-expressing tumors (Redhead and Bailey 2007) to assess whether the presence of PRDM9 binding sites in SVBSs was dependent on its gene expression. This analysis identified a motif enriched in SVBSs from *PRDM9*-expressing samples, which partially matched the motif recognized by PRDM9 (Supplemental Fig. S8). We searched for the complete PRDM9 binding motif in the SVBSs containing the enriched motif from samples expressing *PRDM9* and found that 27% of these SVBSs also matched the entire PRDM9 motif with an FDR < 1%.

Highly recombining regions (HRRs) are known to be associated with PRDM9 activity (Baudat et al. 2010; Myers et al. 2010; Parvanov et al. 2010) and binding (Myers et al. 2010), even in cell lines unrelated to meiotic functions (Eram et al. 2014; Altemose et al. 2017). We computed odds ratios to assess whether tumors expressing *PRDM9* showed enrichment in SVs at HRRs (Hussin et al. 2015) relative to nonexpressing tumors (Fig. 3B). Significant enrichment of SV breakpoints in HRRs of samples expressing *PRDM9* were observed in brain glioblastoma, renal cell cancer, kidney chromophobe, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, and breast cancer (Fig. 3B). In these cancer types, we further determined whether there was an enrichment of SV breakpoints in regions associated with PRDM9-specific H3K4me3 marks. To do so, we leveraged regions marked with H3K4me3 that are known to be attributable to PRDM9 binding, following its transfection in HEK293T cell lines (Altemose et al. 2017). In PRDM9-specific H3K4me3 regions, we observed a significant enrichment of SV breakpoints in samples expressing *PRDM9* in the cancer types having a significant enrichment of breakpoints in HRRs (OR = 1.12 with 95% confidence interval [1.05–1.19], $P = 4.7 \times 10^{-4}$), suggestive of an association between sites of PRDM9 epigenetic activity and SV breakpoints. Potential confounders are *Alu* and *THE1* elements within HRRs, which often contain PRDM9 binding motifs (Myers et al. 2010) and are known to be prone to somatic structural alterations owing to their repeti-

tive structure (Zhang et al. 2011). After excluding SVs with *Alu* and *THE1* elements, similar results were observed (Supplemental Fig. S9). This trend was observed across all SV classes (Supplemental Fig. S10), although some failed to achieve statistical significance due to reduced N, denoting that no specific class of SVs drove the observed association.

Together, our results indicate that for multiple cancer types there was an association between PRDM9-mediated meiotic recombination sites and the locations of SVs in *PRDM9*-expressing cancers. On the other hand, ovarian cancer, lung squamous cell carcinoma, and uterine corpus endometrial carcinoma showed the opposite effect, in which SVs were modestly but significantly depleted at meiotic HRRs (OR < 1) (Fig. 3B) in samples expressing *PRDM9*, implying that other mechanisms may be targeting these regions (Supek and Lehner 2015). Because previous reports suggest that the location of somatic alterations are associated with heterochromatin (Makova and Hardison 2015), we assessed whether there was a difference in the distribution of SV breakpoint sites falling in regions of accessible chromatin in cancer types with HRR versus non-HRR enrichments. By leveraging DNase hypersensitivity data from tissues corresponding to the examined cancer types in the ENCODE Project (ENCODE Project Consortium 2012), we did not detect significant differences in the proportion of SV breakpoints falling in regions of open chromatin (Mann-Whitney *U* test: P -value = 0.6286). This suggests that chromatin architecture of the corresponding tissue alone cannot explain the distribution of SV breakpoint sites. Of these cancer types, the ovarian cancer and uterine corpus endometrial carcinoma cohorts were enriched for the mutational signature of LOF of the homologous recombination repair pathway, leading to an increase of DSBs through the nonhomologous DSB machinery. The enrichment of this mutational signature in ovarian cancer and uterine corpus endometrial carcinoma paired with the depletion of SVs in HRRs raises the possibility that DSBs are less accessible to the nonhomologous DSB machinery in non-HRRs, increasing DSBs unrelated to PRDM9.

Discussion

The function of PRDM9 in meiosis and in recombination is becoming increasingly clear (Walker et al. 2015; Grey et al. 2017; Imai et al. 2017), but its role in cancer still remains ill defined. While *PRDM9* expression has previously been observed in some cancer cell lines, in five ovarian carcinoma samples, and in a single lung adenocarcinoma (Feichtinger et al. 2012), we show an aberrant expression of *PRDM9* among human tumors in vivo in 32 different cancer types and report SV breakpoint enrichments at PRDM9 sites of binding and activity, providing evidence of potential mechanisms through which it may play a role in cancer biology. Previous studies have identified a correlation between acquired SV breakpoints and sites of meiotic crossovers, across recurrent breaks in cohorts of retinoblastomas (Hagstrom and Dryja 1999) and colorectal cancer (Howarth et al. 2009), as well as within individual meiotic and somatic recombination maps (Paulsson et al. 2011). Hussin et al. (2013) and Woodward et al. (2014) postulated that specific PRDM9-defined sites of meiotic recombination in parents possibly increase genomic instability of the offspring, creating genetic susceptibility to cancer in pediatric cases. Furthermore, following the observation of *PRDM9* expression in cancer, Feichtinger et al. (2012) hypothesized this expression may be involved in chromatic lesions.

Our study provides evidence explaining the previously observed enrichment of somatic breakpoints at sites of meiotic

Figure 3. Structural variant (SV) breakpoints are enriched at sites of PRDM9 binding and activity. (A) Proportion of SV breakpoint sequences (SVBSs) significantly matching the motifs recognized by proteins in the JASPAR database, in cancer samples expressing PRDM9. We focused on sequences located within 100 bp of SV breakpoints, which is the mean distance separating DSBs and PRDM9 binding sites in meiosis (Baker et al. 2014). Each row shows the proportion of SVBSs matching the given motif per cancer type. The far-right column shows the proportion of SVBSs matching each motif across all cancer types. Supplemental Figure S7 shows the robustness of these results to significance thresholds used (Methods). (B) Enrichment for SV breakpoints at sites of highly recombining regions (HRRs) in samples expressing PRDM9, as shown by odds ratios >1 for each cancer type. Significant cancer types are shown in blue, as determined using Fisher's exact test ($P < 0.05$ with Bonferroni correction for the number of cancer types tested). Brain glioblastoma multiforme, kidney chromophobe, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, and breast cancer samples all exhibited significant associations between PRDM9 expression and the colocalization of SV breakpoints and meiotic recombination hotspots. Ovarian cancer, lung squamous cell carcinoma, and uterine corpus endometrial carcinoma showed odds ratios <1, indicating significant enrichment for SV breakpoints in non-HRRs in samples expressing PRDM9.

recombination: We observe enrichment of somatic breakpoints at sites of PRDM9 binding and activity. Multiple cancer types, including glioblastoma multiforme, renal cell, kidney chromophobe, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, and breast, all exhibited consistent associations between PRDM9 expression and the colocalization of SV breakpoints with PRDM9's binding and active sites. In these cancers, assuming PRDM9 transcripts are translated into proteins, PRDM9 binding may lead to the recruitment of the DSB machinery. Although genes coding for SPO11, MRE11, and RAD50, which are known to be recruited by PRDM9 for the creation of meiotic DSBs, were not consistently coexpressed with PRDM9 in the surveyed cancers, other coexpressed genes are relevant to the creation of somatic DSBs: The gene coding for the meiotic structure-specific endonuclease EME1, as well as the gene coding for the meiotic recombination protein DMC1, were overexpressed in cancers expressing PRDM9. Furthermore, our detection of the differential expression of DMC1 and EME1 in cancers expressing PRDM9 only when we incorporated sex as a covariate suggests sex bias in PRDM9 downstream interactors in a cancer context, and possibly in a meiotic context as well. The gene coding for the meiotic cohesin REC8, a component of the synaptonemal complex, was also overexpressed in cancers expressing PRDM9. REC8 and PRDM9 interact during meiosis, leading to the displacement of PRDM9-bound recombination hotspots toward the chromosomal axis (Parvanov et al. 2016). In cancers where PRDM9 and REC8 were overexpressed, the interaction between PRDM9 and REC8 may contribute to the disruption of the mitotic chromosomal architecture, possibly leading to SVs. Replication-based mechanisms (Yang et al. 2013) may also explain a subset of somatic SVs: PRDM9 binding might cause a replicative delay leading to increased SV breakpoints. Under this model, however, we would expect binding sites of other proteins to be enriched in SVBs. For example, the expression of PAX5 is restricted to the spleen and to the small intestine in healthy tissues, as shown by the GTEx project (Melé et al. 2015), but was expressed at a threshold >10 FPKM-UQ in 1288 cancers in PCAWG originating from neither the spleen nor the small intestine. Even so, we did not find an enrichment of the binding site recognized by PAX5 in SV breakpoint sequences in samples expressing PAX5 relative to samples that do not express it.

Among cancer types where the evidence for PRDM9 having a role in the genomic localization of SVs was weaker, other exogenic or replication-related mechanisms may be leading to SVs (Glodzik et al. 2017), drowning out signals of PRDM9's contribution. The relationship between aberrant PRDM9 expression and increased SV breakpoints at sites associated with PRDM9 activity is nonlinear: In this study, cancer types with consistent associations between PRDM9 sites of activity and the location of SV breakpoints were not those with the highest PRDM9 expression, raising the possibility that PRDM9-related SVs may be balanced by up-regulation of repair pathways in the same tumors. Furthermore, because PRDM9 binding site locations are dependent on the allele expressed, investigating whether there is a shift in the location of SVs in cancers expressing PRDM9 in populations where the A allele is less frequent may lead to a better understanding of the mechanisms involved in the generation of SVs in cancers.

In contrast with its functional role in the recruitment of the DSB machinery at sites of recombination, little is known about upstream mechanisms associated with PRDM9 expression. We observed that aberrant PRDM9 expression in tumors was correlated with the up-regulation of M1AP expression. In mice, M1ap-knock-down males exhibit meiotic arrest as early as during the zygotene/

pachytene stage (Arango et al. 2013), and missense variation in PRDM9 in humans has been implicated in male infertility (Miyamoto et al. 2008; Irie et al. 2009). The similarity in downstream effects of LOF of both PRDM9 and M1AP, coupled with the observed association between M1AP up-regulation and PRDM9 expression in tumors, suggests a role for M1AP regulating PRDM9 expression during meiosis. We also identified recurrently mutated genomic regions associated with PRDM9 expression, specifically in a region within 5 Mb of the PRDM9 locus. These mutations in the tumor genome potentially point to cis-acting regulatory domains that have been disrupted. The presence of genes having a testis-specific expression neighboring the recurrently mutated regions may also suggest that these somatic mutations disrupt the repression of entire topologically associated domains associated with testis- and meiotic-specific function, including PRDM9.

DSBs generating somatic SVs are a mechanism that contributes to genome instability, which conduces tumorigenesis. Our work provides strong evidence for a novel mechanism underlying genomic instability during tumorigenesis: that aberrant expression of PRDM9 is associated with somatic SVs, raising the intriguing possibility that there are as-yet uncharacterized genomic features and binding sites that lead to SVs. In the future, identification of features that are overrepresented near somatic breakpoints will provide a more complete picture of the endogenous processes associated with genomic instability.

Methods

Data

We retrieved RNA-sequencing samples from 1256 tumors, of which 162 had matched healthy tissue RNA-sequenced (May 2016 version 1.1) from the PCAWG project (<https://dcc.icgc.org/pcawg>). Paired healthy and tumor transcriptome samples were retrieved from the TCGA Research Network ($n=651$ pairs) (<http://cancergenome.nih.gov/>). TCGA samples already present in the PCAWG cohort were discarded. An additional 8555 RNA-sequencing samples from healthy tissue were retrieved from the GTEx Consortium (version 6p) (Melé et al. 2015). Sample distributions across cancer types are shown in Supplemental Figure S1. Raw sequencing FASTQ files were aligned with STAR (Dobin et al. 2013) using pipelines available at https://github.com/ucscCancer/icgc_rnaseq_align. Our conclusions would not be significantly altered through realignment of the data on the most recent version of the reference genome: Our results focus on genomic regions that are not targeted by the major improvements between GRCh37 and GRCh38. SVs were identified by the PCAWG-6 group using the intersect of three different pipelines (Campbell et al. 2017; Li et al. 2017; Yung et al. 2017): The Sanger pipeline used BRASS (<https://github.com/cancerit/BRASS>), the DKFZ/EMBL pipeline used DELLY (Rausch et al. 2012), and the Broad pipeline used SNOWMAN (<https://github.com/broadinstitute/SnowmanSV/>) and DRanger (Supplemental Fig. S6; <http://www.broadinstitute.org/cancer/cga/dranger>). Details on the calling of consensus SVs is detailed in the PCAWG-6 marker paper (Li et al. 2017).

RNA-sequencing data quantification and normalization

RNA-sequencing was quantified for each transcript using HTSeq (version 0.6.1p1) (Anders et al. 2015) over all gene IDs from a modified version of the GENCODE v19 annotation file that only considered the PRDM9 locus to span the PRDM9/PRDM7 low-homology region. Overlaps were resolved using the intersection of all nonempty sets. Read counts for each gene were then

normalized into FPKM-UQ, which gives a more uniform distribution across genes than FPKM (Bullard et al. 2010).

PRDM7 homology correction

Owing to the high homology between *PRDM9* and its paralog *PRDM7* (Supplemental Fig. S2A; Fumasoni et al. 2007; Blazer et al. 2016), we used the PCAWG cohort to evaluate a homology correction based on low-homology regions of the two genes to ensure that the computed *PRDM9* read counts actually originated from *PRDM9* transcripts. This homology correction added the contribution of a read toward the *PRDM9* read count only if it mapped uniquely to a restricted 756-bp region of low homology between *PRDM9* and *PRDM7*, namely, the ZnF array domain of *PRDM9* (hg19; Chr 5: 23,526,838–23,528,706). Upon comparing computed *PRDM9* expression levels before and after applying the homology correction within each sample (Supplemental Fig. S2B), we found that the two metrics were highly correlated (Pearson's correlation coefficient = 0.98, $P < 2.2 \times 10^{-16}$), although 246 samples expressed *PRDM9* above a threshold of 10 FPKM-UQ only in the uncorrected samples. Among reads mapping to the *PRDM9* locus, there were no *PRDM7*-specific substitutions in the SET domain. We interpreted these 246 samples to be false positives due to homologous *PRDM7* expression or to other mapping biases and removed these samples in further analyses. It is also highly likely that the homology correction underestimated the expression levels of *PRDM9* due to the repetitive nature of the ZnF array, which encompasses most of the low-homology region (Hinch et al. 2011; Hussin et al. 2013).

Comparisons of *PRDM9* expression between cohorts

Within analyses including only the PCAWG and TCGA data sets, samples were considered to express *PRDM9* if the FPKM-UQ for this gene was above a threshold of 10. In analyses including the GTEx data, we considered a more stringent expression threshold of 50 FPKM-UQ to account for higher levels of technical variation across different cohorts. We tested for differences in *PRDM9* expression between matching tumor and surrounding healthy tissue from the TCGA and PCAWG cohorts with a one-sided Wilcoxon signed-rank test. We applied this same test for pairs of samples per cancer type and used Fisher's method to combine *P*-values from multiple test statistics, employing the metap R package (version 0.8) (<https://CRAN.R-project.org/package=metap>). We used a one-sided two-sample test for equality of proportions to test for differences in samples expressing *PRDM9* between the healthy GTEx cohort, excluding testis samples, and the tumor PCAWG cohort.

DGE analysis

The DGE analyses were performed using DESeq2 (Love et al. 2014). Only genes that had a mean of 10 counts across all samples were considered, resulting in a total of 23,413 genes assessed. We considered samples with FPKM-UQ > 10 to express *PRDM9* and samples with FPKM-UQ = 0 to not express *PRDM9*, both in the corrected and in the uncorrected analyses, resulting in 209 samples expressing *PRDM9* and 907 that did not. The PCAWG project codes, representing the cancer type and country of origin of different cancer cohorts, were included as a cofactor to account for tissue- and cohort-specific differences in expression. Reported *P*-values were adjusted to assess the false-discovery rate (FDR) using the Benjamini and Hochberg procedure (Benjamini and Hochberg 1995). By using these adjusted *P*-values, we determined significance if they fell below a prescribed threshold for FDR (here, <1%), as well as a log₂ fold change >2 in gene expression. For DGE analyses including sex as a cofactor, sex-specific cancer types were not considered. Gene lists

associated with the meiosis biological process (GO:0051321) were extracted using QuickGO (Huntley et al. 2015). The gene list enriched in testes was obtained from the Human Protein Atlas (Uhlén et al. 2015). Modules represent highly interacting gene products of differentially expressed genes associated with *PRDM9* expression and were defined using the Reactome FI for Cytoscape (Wu et al. 2010). Modules shown included at least 50 nodes in differentially expressed genes and nine nodes in genes enriched in testes. Enriched GO biological processes in each module were defined as having an FDR <5%.

Association between *PRDM9* expression and recurrently mutated regions

Recurrently mutated regions are defined as densely mutated regions where at least two SNVs across all tumor samples were located within 100 bp from each other, as described by Lamaze et al. (2017). We tested for linear regression between *PRDM9* expression and each recurrently mutated region disrupted in at least 5% of samples, accounting for cancer type. Significance was established with $P < 0.05$, corrected using a Bonferroni correction for multiple testing for each recurrently mutated region tested ($n = 5548$). Genes closest to each significant recurrently mutated region were assessed using BEDTools closest (version 2.25) (Quinlan 2014), using the hg19 Ensembl gene list as a reference. Testis-specific expression of genes closest to significant recurrently mutated regions located on Chromosome 5 were manually inspected in the GTEx data portal.

PRDM9 binding motif analyses

There is variation in *PRDM9* binding sites within and across individuals carrying different alleles. To uncover a binding site robustly recognized by the A-variant of *PRDM9*, we used MEME-ChIP from the MEME package (Bailey et al. 2009) to identify a motif shared among sites of DSBs in a previous study using sperm from individuals genotyped as AA (Pratto et al. 2014). SVBSs were identified using BEDTools slop (version 2.25) (Quinlan 2014) by adding 100 bp flanking each SV breakpoint, because this is the approximate distance separating DSBs and *PRDM9* binding sites in meiosis (Baker et al. 2014). Motifs enriched in these sequences were identified by a comparison with the JASPAR core vertebrate database (version 2016) (Mathelier et al. 2016), using the R package rtfbs (version 0.3.5). SVBSs were partitioned into four categories based on GC content to reduce the number of false positives. Enrichments were tested by comparing motif occurrence in SVBSs to that in random sequences matched for GC content, and significance was assessed using FDR thresholds of 0.1 and 0.01. Results shown only include human binding sites and are the 25 motifs with the highest proportion of SVBSs matching each respective motif across all cancer types.

To test whether the high number of significant *PRDM9* motif matches overlapped with SVBSs were specific to samples expressing *PRDM9*, we performed a discriminative regular expression motif search using DREME from the MEME package (Bailey et al. 2009), which searches for motif patterns that can discriminate between two sets of sequences. The primary set of sequences consisted of SVBSs found in samples expressing *PRDM9*, while the negative control set of sequences consisted of SVBSs identified in samples without *PRDM9* expression, both before and after the *PRDM7* homology correction. From these results, we searched for the complete *PRDM9* binding motif in sequences containing the discriminatory motif enriched in SVBSs from samples expressing *PRDM9* using FIMO from the MEME package (MEME/4.9.1_1).

Odds ratios calculations

HRRs of the genome were determined as defined by Hussin et al. (2015). The number of SV breakpoints falling in HRRs and in the rest of the genome was determined using BEDTools intersect (version 2.25) (Quinlan 2014). Odds ratios were computed to quantify the differences in proportions of SVs falling in the HRRs relative to the rest of the genome (Non_HRR). For each cancer type, the odds ratios were computed as $OR = (x_{HRR} \times y_{Non_HRR}) / (x_{Non_HRR} \times y_{HRR})$, where x_{HRR} and x_{Non_HRR} represent the number of SVs in samples with PRDM9 expression, and y_{HRR} and y_{Non_HRR} represent the number of SVs in samples that do not express PRDM9. Ninety-five percent confidence intervals were computed as detailed by Szumilas (2010), and significance was established using a Fisher's exact test, with a $P < 0.05$ threshold after Bonferroni correction for multiple testing for the different cancers tested. PRDM9-specific H3K4me3 marked regions were identified from ChIP-seq data from Altomose et al. (2017). H3K4me3 peaks in PRDM9-transfected cells (GEO sample accession: GSM2643614) that did not overlap any peaks from both replicates of untransfected cells (GEO sample accession: GSM2643608 and GSM2643609) were considered as PRDM9-attributable H3K4me3 peaks.

The locations of *Alu* and *THE1* repeat elements were extracted from the hg19 RepeatMasker (<http://www.repeatmasker.org>) track on UCSC's genome browser. SV breakpoints and HRRs overlapping *Alu* and *THE1* elements were discarded, and then odds ratios were computed. SV classification was done based on results provided by the PCAWG consortium (Li et al. 2017). Odds ratios, significance, and confidence intervals were determined as previously described for each SV type.

DNase-seq data were retrieved as BED files (broad peaks) from the ENCODE data portal (ENCODE Project Consortium 2012). The tissue correspondences used were as follows: Brain glioblastoma multiforme, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, breast cancer, ovarian cancer, lung squamous cell carcinoma, and uterine corpus endometrial carcinoma were matched, respectively, with cerebellum and frontal cortex, esophagus squamous epithelium, right lobe of liver, breast epithelium, ovary, upper lobe of left lung, and uterus tissue types.

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Aberrant *PRDM9* expression impacts the pan-cancer genomic landscape

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