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Publication Date

2016-12-07

DOI

10.1038/srep38133

Peer reviewed

SCIENTIFIC REPORTS



OPEN

Activation induced deaminase mutational signature overlaps with CpG methylation sites in follicular lymphoma and other cancers

Received: 01 July 2016
Accepted: 07 November 2016
Published: 07 December 2016

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Follicular lymphoma (FL) is an incurable cancer characterized by progressive severity of relapses. We analyzed sequence context specificity of mutations in the B cells from a large cohort of FL patients. We revealed substantial excess of mutations within a novel hybrid nucleotide motif: the signature of somatic hypermutation (SHM) enzyme, Activation Induced Deaminase (AID), which overlaps the CpG methylation site. This finding implies that in FL the SHM machinery acts at genomic sites containing methylated cytosine. We identified the prevalence of this hybrid mutational signature in many other types of human cancer, suggesting that AID-mediated, CpG-methylation dependent mutagenesis is a common feature of tumorigenesis.

Analysis of the numerous mutations present in cancer genomes is expected to substantially contribute to our understanding of the causes of malignancy and eventually to the development of personalized treatment plans. DNA sequence contexts of mutations in tumors can provide insights into the mechanisms of mutagenesis in cancer^{1–3}. The ‘mutational signature’ approach was introduced in the 1990s^{4–6} and has been successfully applied to delineate the roles of AID and DNA polymerase η in somatic hypermutation in humoral immunity^{5,7,8}, editing APOBEC3s cytosine deaminases in hypermutagenesis in retroviruses⁹ and the formation of dimers versus 6–4 photoproducts in UV- mutagenesis¹⁰. Recently, this methodology has become popular in the analysis of cancer genomes^{3,11}. As predicted after the discovery of DNA editing by AID and APOBEC cytosine deaminases¹², mutations in DNA sequence contexts similar to mutations induced by deaminases in model systems have been found in several types of cancer^{2,3,13,14}. Studies of mutations induced by deaminases are facilitated by their unique properties, namely, the ability to produce, *in vitro*, clustered mutations in ssDNA at specific contexts surrounding cytosines^{8,15,16} and retention of the signatures of deaminase-induced mutagenesis and propensity for clustered mutations, kataegis *in vivo*, in heterologous models where no potential specific cofactors are expected to be present^{17–23}.

Based on DNA sequence context and other approaches, it has been shown that AID, which generates mutations of C-G pairs in the WRC/QYW motif (Fig. 1, upper row, mutated base pair is underlined), could contribute to gastric and haemopoietic cancers^{24,25}, whereas APOBEC3A (A3A) and A3B (TCW/WGA motif, Fig. 1, fourth row) potentially contribute to breast, lung and many other cancers^{17,26–28}. A recent report indicates that deaminase-induced clusters of mutations mark signatures of accelerated somatic evolution in cancer gene promoters in lymphoma²⁹. Mutational signatures are critical in the analysis and are subject to continuous refinement³⁰. Here we describe a novel, unexpected mutational signature of AID deaminase that is linked to DNA CpG

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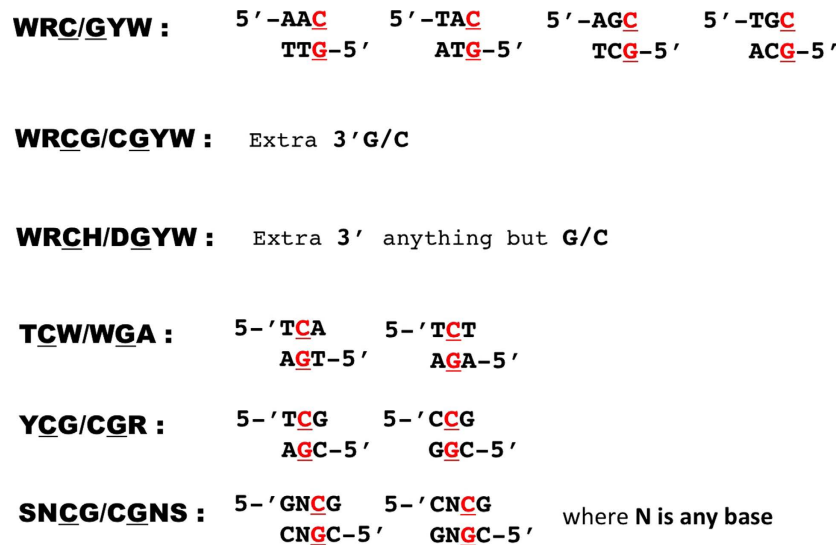


Figure 1. Mutable DNA sequence motifs analyzed in this work. Variants of DNA sequences corresponding to a defined motif (left column, bold) are shown to the right in the double-stranded DNA form. Mutation-prone bases are in red and underlined.

methylation. We initially identified the hybrid signature in follicular lymphoma and then in more than a half of all types of human cancers.

Results and Discussion

We analyzed over 13,000 base substitutions found in follicular lymphoma (FL) in 22 patients (Supplemental Table 1). Mutations at G-C base pairs were 1.5 times more frequent than mutations at A-T pairs; the number of transversions was approximately equal to the number of transitions. The overall pattern of base substitutions in FL has similarities both to the classic distribution of types of changes during spontaneous mutagenesis in humans³¹ and to somatic hypermutation of immunoglobulin genes⁷ (Supplemental Fig. 1). However, the FL mutational spectrum showed alterations in the ratios of transversions in G-C pairs, namely a two-fold relative increase in the fraction of G-C to T-A and a two-fold decrease in the fraction of G-C to C-G transversions, which could be a sign of modulation of processes of DNA damage and translesion DNA synthesis at G-C pairs³².

Examination of the DNA sequence context of mutations in FL showed that the bias was caused by a significant excess of substitutions in CpG dinucleotides, with the implication that the mechanism of these mutations is linked to cytosine methylation/demethylation^{33,34}. Briefly, the analysis was performed as follows. We calculated the excess of mutations in specific motifs using the ratio F_m/F_n , where F_m is the fraction of mutations observed in the particular motif, and F_n is the frequency of the motif in the respective DNA neighborhood (defined as a 120 bp DNA sequence window, Supplemental Dataset S1). A 2.3-fold excess of mutations (defined as described in Materials and Methods) in **CG/CG** dinucleotides was detected (Table 1, row 1). In contrast, there was no association between mutations and the **TCW/WGA** motif, indicating that APOBEC1 and APOBEC3 are not involved in mutagenesis in FL (Table 1, row 2). Instead, we detected the signatures of AID and of Pol η (Table 1, rows 3–6), which are known as mutators involved in immunoglobulin genes somatic hypermutation (SHM) at G-C and at A-T base pairs, respectively³⁵. Unexpectedly, however, the most strongly over-represented motif was **WRCG/CGYW**, which is a combination of the AID motif **WRC/GYW** and the CpG dinucleotide; in contrast, no connection between **WRC/GYW** and somatic mutations was found in non CpG sites when CpG was masked (Table 1, last three rows). Notably, SHM in immunoglobulin genes shows the opposite trend whereby somatic mutations are substantially underrepresented in CpG-containing motifs³⁶. Thus, the mutational process in FL appears to be distinct from the conventional SHM and is likely associated with CpG methylation/demethylation processes. AID deaminates 5-methylcytosine in characteristic AID-target sequence contexts, and the footprint of AID-induced mutagenesis has been found in oncogenes mutated in tumours³⁷. Deamination of methylated cytosines by AID and APOBECs³⁸ is thought to contribute to a variety of genetic and epigenetic processes^{39–42}, which potentially could be compromised in FL cells, leading to AID-dependent mutagenesis.

The only deviation from this novel mutation pattern in FL was found in 5'UTRs where SHM appears to operate in the “standard immunoglobulin mode” (significant correlation of mutation context with **WRCH/DGYW** and **WA** motifs, Supplemental Table 2). Although elevated mutagenesis was observed in CpG dinucleotides and **CGYW** motif similar to other gene regions, the two processes did not overlap and the hybrid signature was not detected. The 5'UTRs are known to be preferentially targeted by deaminases in active genes^{43–45}, therefore the hybrid motif might be masked by numerous AID and other deaminases-induced mutations.

We analyzed AID-related **WRC/GYW** and **WRCG/CGYW** motifs for 22 individual FL patient exomes (Supplemental Table 3). A significant excess of both motifs was found for 13 patients. This finding suggests that the mutational processes associated with AID are active in FL to the extent detectable with sensitive statistical tests in samples with limited number of mutations. To determine whether the observed excess of **WRCG/CGYW**

Mutable motif	Mutator protein/system	Reference	Excess of mutations in the motif	Type of statistical test*	P-value**
<u>C</u> G/ <u>C</u> G	CpG methylation	33	2.3	Fisher	<10 ⁻⁸
				Monte-Carlo	<0.001
<u>T</u> C <u>W</u> / <u>W</u> G <u>A</u>	APOBEC1 (A1) or A3A or A3B	27,28,55	0.9	Fisher	NS
				Monte-Carlo	NS
W <u>R</u> C/ <u>G</u> Y <u>W</u>	AID <i>in vitro</i>	8	1.2	Fisher	<10 ⁻⁷
				Monte-Carlo	<0.001
W <u>R</u> C <u>H</u> / <u>D</u> C <u>G</u> Y <u>W</u>	AID component of somatic hypermutation <i>in vivo</i> (SHM/AID)	56	1.0	Fisher	NS
				Monte-Carlo	NS
W <u>R</u> C <u>G</u> / <u>C</u> G <u>Y</u> W	SHM/AID in FL	This study	2.7	Fisher	<10 ⁻⁸
				Monte-Carlo	<0.001
W <u>A</u> / <u>T</u> W	DNA polymerase η (pol η)	7,57	1.1	Fisher	<2 × 10 ⁻⁵
				Monte-Carlo	<0.001
<u>All CpG dinucleotides were masked in studied sequences</u>					
<u>T</u> C <u>W</u> / <u>W</u> G <u>A</u>	A1/A3A/A3B		0.8	Fisher	NS
				Monte-Carlo	NS
W <u>R</u> C/ <u>G</u> Y <u>W</u>	AID <i>in vitro</i>		0.9	Fisher	NS
				Monte-Carlo	NS
W <u>A</u> / <u>T</u> W	pol η		1.1	Fisher	<2 × 10 ⁻⁵
				Monte-Carlo	<0.001

Table 1. Association between known mutable motifs and the DNA sequence context of somatic mutations in exomes of follicular lymphoma. *The correlation was measured using Fisher exact test (Fisher) and Monte Carlo (MC) test. Mutable positions in consensus sequences are underlined (R = A or G; Y = T or C; M = A or C; K = G or T; D = A, T or G; H = A, T, or C; W = A or T, Fig. 1). The excess of mutations in motifs was calculated using the ratio Fm/Fn, where Fm is the fraction of somatic mutations observed in the given mutable motif (the number of mutated motifs divided by the number of mutations), and Fn is the frequency of the motif in the DNA neighborhood of somatic mutations (the number of motif positions divided by the total number of all un-mutated positions in the 120 bp window). **NS, no significant excess.

motifs could be a simple consequence of an extremely high mutability of CpG dinucleotides, we compared the relative frequencies of mutations in the WRCG/CGYW motifs and in CpG-containing contexts that do not contain the WRC/GYW motif, namely YCG/CGR and SNCG/CGNS, in different cancer cell lines. In FL and in many other cancers, there was a highly significant excess of mutations in WRCG/CGYW compared to the motifs lacking WRC (Table 2) indicating that the overlap of the AID motif and CpG indeed is the unique mutagenesis signature. In a diverse collection of cancer genomes, we found a significant excess of WRCG/CGYW motifs in two distinct types of blood cancer with the highest representation in the COSMIC data set, as well as in 9 out of 14 analyzed solid tumors from various tissue types, particularly in stomach cancer. Among tissues without an excess of mutated WRCG/CGYW motif, skin has an exceptionally low rate of mutations in this motif, consistent with the previous observations that a different motif (YCG/CGR) is hypermutated in human skin cancers^{46,47}. Importantly, the signatures characteristic to AID activity are detectable specifically in cancer genomes. For control, we examined the context of somatic mutations in various normal tissues⁴⁸ and did not find any significant excess of AID-related mutable motifs, either CpG-containing or not (Supplemental Tables 4 and 5). The size of these datasets are limited, but power analysis (Materials and Methods) suggested that the absence of any significant excess of AID-related mutable motifs likely reflects genuine biological properties of these samples.

The striking abundance of mutations in WRCG/CGYW motifs in tumors implies that AID is sufficiently active in many human cancer types to skew the mutation distribution towards the AID WRC/GYW motifs. These observations are in line with the previous findings on the involvement of AID in gastric cancers²⁵ and the growing evidence on the role of AID in CpG demethylation in some genomic regions^{40,49}. We analyzed the mutability of WRC/GYW motifs in various cancer genomes from COSMIC and observed that almost half of the cancer types (6 of the 16) show a significant excess of mutations in these motifs (Table 3). The high mutation prevalence in the “pure” AID motif strongly correlates with that in the hybrid “AID and CpG” motif across the range of cancers. However, the apparent correlation is not perfect and the excess of mutations in WRC/GYW is generally weaker (Fig. 2). The cancers without excess of mutations in WRCG/CGYW (breast, bladder, cervix, lung, skin) show no increased mutability of the WRC/GYW motif either. The difference in the mutability patterns between the two motifs in part can be explained by the greater statistical power of the more informative WRCG/CGYW motifs compared to WRC/GYW motifs. When the involvement of AID is not supported at a statistically significant level through the WRC/GYW motif, it might still act at CpG dinucleotides causing a significant deviation from the expected mutation frequencies for the WRCG/CGYW motif.

Tissue/cancer	Fraction mutated WR \underline{C} G/CGY \underline{W} ** (total number of motifs)	Fraction mutated Y \underline{C} G/C \underline{G} R and SN \underline{C} G/ CGNS** (total number of motifs)	P-value, Fisher test
Follicular lymphoma	0.046 (9,438)	0.039 (37,315)	0.005
Blood	0.063 (16,668)	0.050 (71,551)	$<10^{-10}$
Blood: Acute Myeloid Leukemia	0.067 (10,226)	0.048 (43,958)	$<10^{-10}$
Blood: GCB Lymphomas	0.063 (5,033)	0.050 (21,674)	5×10^{-5}
<u>Breast</u>	0.037 (118,009)	0.037 (449,770)	NS
<u>Bladder</u>	0.022 (55,861)	0.029 (221,217)	NS
<u>Cervix</u>	0.02 (58,896)	0.029 (231,074)	NS
Colon	0.102 (261,993)	0.073 (1,018,315)	$<10^{-10}$
Kidney	0.039 (45,835)	0.032 (179,222)	$<10^{-10}$
Liver	0.036 (115,921)	0.030 (480,198)	$<10^{-10}$
<u>Lung</u>	0.036 (221,301)	0.036 (872,132)	NS
Ovary	0.044 (33,436)	0.037 (132,132)	$<10^{-10}$
Pancreas	0.077 (60,409)	0.057 (242,824)	$<10^{-10}$
Prostate	0.072 (27,084)	0.064 (106,577)	3×10^{-5}
Rectum	0.086 (46,433)	0.068 (177,479)	$<10^{-10}$
<u>Skin</u>	0.004 (201,866)	0.042 (824,249)	NS
Stomach	0.090 (204,610)	0.061 (802,363)	$<10^{-10}$
Uterus	0.054 (84,699)	0.046 (317,894)	$<10^{-10}$

Table 2. Difference between the mutability of AID motifs WR \underline{C} /G \underline{Y} W with vs without an extra 3' GC pair (Fig. 1) in various cancer genomes (the Sanger COSMIC Whole Genome Project)*. Tissue types without significant correlation (taking into account the Bonferroni correction for multiple tests) between the motif and somatic mutations are underlined. "Fraction mutated C \underline{G} Y \underline{W} " and "Fraction mutated Y \underline{C} G/C \underline{G} R and SN \underline{C} G/CGNS" are fractions of mutated motifs (the number of the mutated motifs divided by the total number of motifs in the analyzed data set). Absence of significant excess of mutations in C \underline{G} Y \underline{W} /WR \underline{C} G (NS, no significant excess) indicates that there is no connection between mutagenesis of C \underline{G} and G \underline{Y} W motifs. **Total number of motifs are in brackets.

We next compared the expression levels of the AICDA gene, which encodes AID, between the TCGA cohorts. Quartiles and extrema were calculated for each TCGA cohort selected in the study (Supplementary Fig. 2). The observed high variability in AICDA gene expression in B-cell Lymphoma (DLBC) is on par with the observation of widely varying levels of AICDA expression in peripheral blood mononuclear cells of patients with B-CLL⁵⁰. The expression levels in all other tumor tissues are within the range where definitive conclusions cannot be made based on the data currently available in TCGA (Supplementary Fig. 2). In most tumor cohorts, however, the quantitative profile of the expression values represented by the five numbers summary (and especially the high variability of AICDA expression; see Supplementary Fig. 2) closely follows the one of B-cell lymphoma, which is consistent with the hypothesis presented here.

We next analyzed mutations and the overall level of methylation (% of methylated cytosines or methylation ratio) for 26 patients with malignant lymphoma (<https://dcc.icgc.org/projects/MALY-DE>, see Methods for details). Consistent with our previous findings (Tables 1 and 3), there is a substantial excess of mutations in WR \underline{C} G/CGY \underline{W} and WR \underline{C} /G \underline{Y} W motifs (4.91 times and 1.53 times, respectively, $P < 10^{-10}$ for both motifs). Analysis of the relative frequencies of mutations in the WR \underline{C} G/CGY \underline{W} motifs and in CpG-containing contexts that do not contain WR \underline{C} /G \underline{Y} W, namely Y \underline{C} G/C \underline{G} R and SN \underline{C} G/CGNS, also revealed a highly significant excess (1.5 times, $P < 10^{-10}$) of mutations in motifs containing AID-mutable WR \underline{C} /G \underline{Y} W, indicating that the overlap of the AID motif and CpG is indeed the signature of mutation process in malignant lymphoma similar to other blood cancers (Table 2). Examination of the association between the methylation ratio and somatic mutations in WR \underline{C} G/CGY \underline{W} mutable motifs identified a moderate but significant decrease of methylation in the WR \underline{C} G/CGY \underline{W} mutation context. The mean methylation ratios for the WR \underline{C} G/CGY \underline{W} mutation positions and non-C \underline{G} Y \underline{W} mutation positions (Y \underline{C} G/C \underline{G} R and SN \underline{C} G/CGNS) were 74.8 and 79.4 respectively ($p < 0.0001$ according to the sampling test; see Methods for details). The histogram in Fig. 3 shows that the major difference is within the range of methylation ratios of 80 and 100, i.e. in mutation positions with large methylation ratios. This finding is consistent with the hypothesis that AID-dependent demethylation preferentially occurs in WR \underline{C} G/CGY \underline{W} mutable motifs so that mutations are one of the outcomes of the multistep demethylation process³⁷. No significant difference between the WR \underline{C} G/CGY \underline{W} mutable motifs and non- WR \underline{C} G/CGY \underline{W} contexts was found for all genomic positions without taking into account somatic mutations in the same set of methylated CpGs (<https://dcc.icgc.org/projects/MALY-DE>, mean values of the methylation ratio are 73.9 and 74.6, respectively) although the slight overall decrease in the methylation ratio in WR \underline{C} G/CGY \underline{W} motifs might have biological implications. These findings are compatible with the hypothesis that AID is involved in demethylation of methylated cytosines during cancer initiation and/or progression.

Tissue	Fraction of mutations observed in the mutable motif	Fraction of motifs in surrounding regions	Excess of mutations in the motif	P-value Fisher exact test
Blood	0.265 (10,633)	0.222 (630,338)	1.19	$<10^{-10}$
Blood: Acute Myeloid Leukemia	0.254 (6,844)	0.221 (405,754)	1.15	$<10^{-10}$
Blood: GCB Lymphomas	0.296 (2,747)	0.221 (165,805)	1.34	$<10^{-10}$
<u>Breast</u>	0.146 (85,203)	0.230 (4,877,914)	0.64	NS
<u>Bladder</u>	0.093 (38,750)	0.229 (2,247,271)	0.41	NS
<u>Cervix</u>	0.074 (41,454)	0.229 (2,399,601)	0.32	NS
Colon	0.257 (175,109)	0.221 (8,986,392)	1.16	$<10^{-10}$
<u>Kidney</u>	0.225 (32,382)	0.228 (1,875,298)	0.99	NS
<u>Liver</u>	0.226 (74,161)	0.222 (4,426,455)	1.02	NS
<u>Lung</u>	0.174 (180,284)	0.226 (9,867,123)	0.77	NS
<u>Ovary</u>	0.229 (22,340)	0.225 (1,309,849)	1.02	NS
Pancreas	0.227 (35,165)	0.220 (2,112,760)	1.03	2×10^{-3}
<u>Prostate</u>	0.22 (13,036)	0.222 (775,226)	0.99	NS
<u>Rectum</u>	0.223 (23,330)	0.226 (1,343,391)	0.99	NS
<u>Skin</u>	0.050 (198,098)	0.224 (8,986,960)	0.22	NS
Stomach	0.274 (115,652)	0.221 (6,977,875)	1.24	$<10^{-10}$
Uterus	0.254 (55,999)	0.229 (3,212,849)	1.11	$<10^{-10}$

Table 3. Preferential mutability of WRC/GYW and somatic mutations in various cancer Whole Genomes and Whole Exomes (the Sanger COSMIC Whole Genome Project)*. Tissue types without significant correlation (taking into account the Bonferroni correction for multiple tests) between the motif and somatic mutations are underlined. The excess of mutations in motifs was calculated using the ratio F_{sm}/F_c , where F_{sm} is the fraction of somatic mutations observed in the studied mutable motif (the number of mutated motifs divided by the number of mutations), and F_c is the frequency of the motif in the DNA context of somatic mutations (the number of motif positions divided by the total number of all un-mutated positions in surrounding regions). *Absence of significant excess of mutations in WRC/GYW (NS, no significant excess) suggests that there is no connection between mutagenesis and WRC/GYW motifs.

The analysis of mutations in cancer genomes presented here shows a cancer-specific AID mutational signature that overlaps with the CpG dinucleotide. Thus, AID mutagenesis linked with methylation/demethylation of CpG appears to be a widespread phenomenon in human cancers. The specific mechanisms of the interaction between the CpG (de)methylation and AID-mediated mutagenesis remain to be elucidated. The broader implication of these findings is that epigenetic effects can be directly relevant for somatic mutagenesis in many if not most cancers.

Methods

The exome sequencing data of 22 follicular lymphoma patients were described previously⁵¹. DNA sequences surrounding the mutated nucleotide represent the mutation context. We compared the frequency of known mutable motifs for somatic mutations with the frequency of these motifs in the vicinity of the mutated nucleotide. Specifically, for each base substitution the 120 bp sequence centered at the mutation was extracted (the DNA neighborhood). We used only the nucleotides immediately surrounding mutations because AID/APOBEC enzymes are thought to scan a limited area of DNA to deaminate (methyl)cytosines in a preferred motif²⁶. This approach does not exclude any given area of the genome in general, but rather uses the areas within each sample where mutagenesis has happened (taking into account the variability in mutation rates across the human genome), and then evaluates whether the mutagenesis in this sample was enriched for AID/APOBEC motifs²⁶. This approach was thoroughly tested and a high accuracy of the analysis was shown²⁶. The frequency of mutable motifs in the positions of somatic mutations was compared to the frequency of the same motifs in the DNA neighborhood (Fig. 1) using Fisher exact test (2×2 table, 2-tail test) and Monte Carlo test (MC, 1-tail test) as previously described^{52–54} (for details see Supplementary Fig. 3). Somatic mutation data from ICGC and TCGA cancer genomic projects were extracted from the Sanger COSMIC Whole Genome Project v75 was downloaded from <http://cancer.sanger.ac.uk/wgs>. The tissues and cancer types where defined according to primary tumor site and cancer projects. Somatic mutations in various normal tissues were from⁴⁸ (Supplementary Table 5).

We compared magnitude of the difference between the fraction of mutations observed in the mutable motif and the fraction of motifs in surrounding region (effect size) for somatic mutations in normal tissues. For the purpose of this comparison (power analysis), we used a sampling procedure that was repeated 1,000 times. Each sample of somatic mutations from blood and stomach cancers (where significant excess of somatic mutations in WRC/GYW motifs was observed, Tables 2 and 3) had the size equal to those for normal tissues (674 for blood and 49 for stomach, Supplementary Table 5). Analysis of the difference between the fractions showed that the difference for normal mutations was smaller for 98.3% blood cancer samples and for 94.7% stomach cancer samples. Thus the observed effect size (Supplementary Table 5) is likely to reflect biological properties of these samples and is unlikely to be a result of the small sample size at least for somatic mutations from blood and stomach.

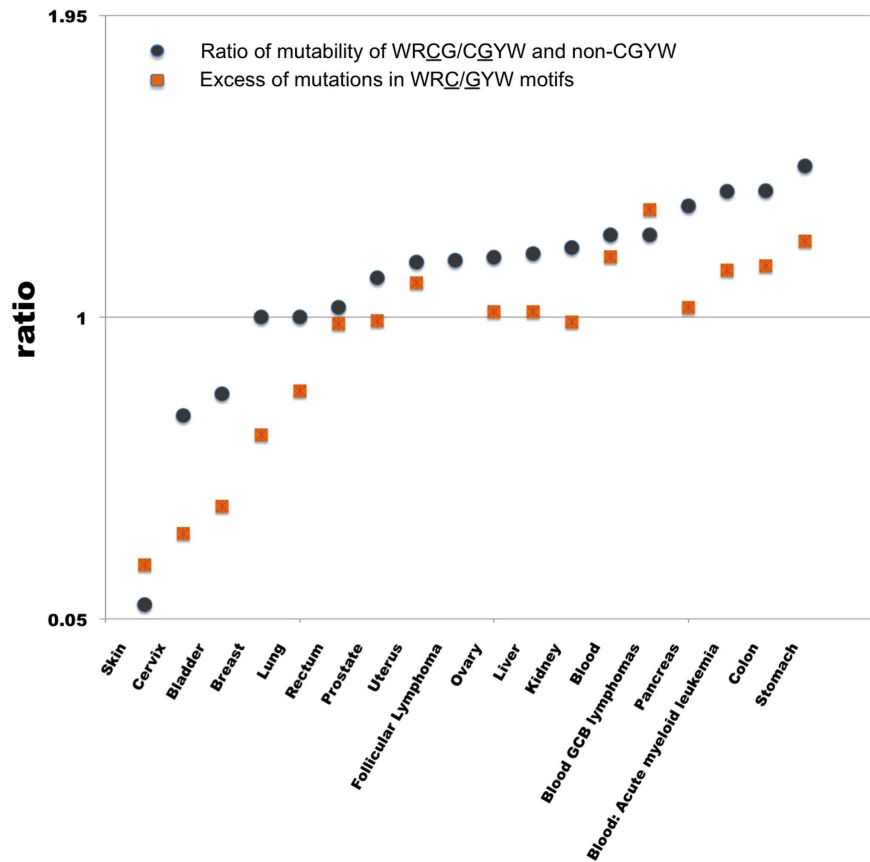


Figure 2. Tumors types with mutation enrichment in the hybrid AID/CpG motif tend to possess an excess of mutations with pure AID signature.

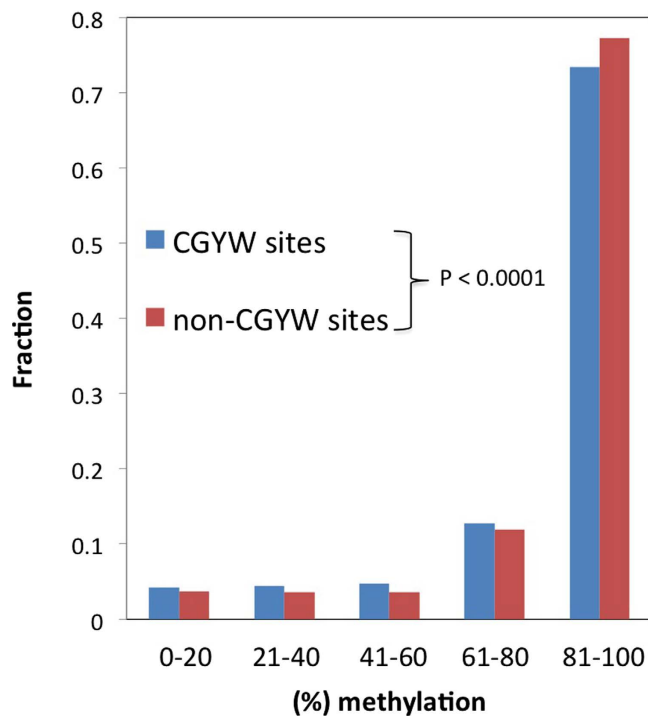


Figure 3. The methylation ratio in WRCG motifs and non-WRCG motifs (YCG/CGR and SNCG/CGNS motifs). The fraction of motifs in each bin (0–20% methylation ratio, 20–40% methylation ratio, etc.) is shown.

For the AICDA gene expression analysis, the normalized version of the RSEM (Broad Institute TCGA Genome Data Analysis Center (2016) Analysis-ready standardized TCGA data from Broad GDAC Firehose 2016_01_28 run. Broad Institute of MIT and Harvard. Dataset. <http://doi.org/10.7908/C11G0KM9>) was used to analyze the TCGA RNA-Seq datasets from the Broad Genome Data Analysis Center. For each TCGA cohort (Supplementary Fig. 2). The low and upper bounds, median, outliers, and first and third quartiles were retrieved via the FireBrowse RESTful API (<http://firebrowse.org/api-docs/>) for the tumor and the corresponding normal (when available) tissue samples.

For the analysis of the association between somatic mutations, mutable motifs (WRCG/CGYW) and methylation, datasets for 26 patients with malignant lymphoma (<https://dcc.icgc.org/projects/MALY-DE>) were used. In the analyzed datasets, the data for all patients were pooled together (the Supplemental Dataset S2 contains the studied set of somatic mutations). Each position is characterized by the methylated/unmethylated read count and the methylation ratio (the number of methylated reads divided by the total number of reads overlapping this position and multiplied by 100). Only positions with more than nine associated reads were included in the analysis. The mean value for mutation positions with (M1) and without WRCG/CGYW (M2) mutable motifs (3620 and 11003 positions, respectively) was calculated. To compare the difference between these two types of positions, methylation ratio values from the larger dataset were randomly sampled until the number of positions was the same as in the smaller dataset. For each sampled dataset, the mean value (M2_sampled) was calculated and the probability $P(M1 \geq M2_sampled)$ was calculated from 10,000 sampled datasets. The same sampling procedure was used for all genomic positions without taking into account positions of somatic mutations. Code availability: A set of *ad hoc* programs is available upon request from Igor B. Rogozin (rogozin@ncbi.nlm.nih.gov).

References

- Alexandrov, L. B. *et al.* Signatures of mutational processes in human cancer. *Nature* **500**, 415–421 (2013).
- Alexandrov, L. B. & Stratton, M. R. Mutational signatures: the patterns of somatic mutations hidden in cancer genomes. *Current Opinion in Genetics & Development* **24**, 52–60 (2014).
- Roberts, S. A. & Gordenin, D. A. Hypermutation in human cancer genomes: footprints and mechanisms. *Nature Reviews. Cancer* **14**, 786–800 (2014).
- Bachl, J., Steinberg, C. & Wabl, M. Critical test of hot spot motifs for immunoglobulin hypermutation. *European Journal of Immunology* **27**, 3398–3403 (1997).
- Rogozin, I. B. & Kolchanov, N. A. Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighbouring base sequences on mutagenesis. *Biochimica et Biophysica Acta* **1171**, 11–18 (1992).
- Rogozin, I. B., Sredneva, N. E. & Kolchanov, N. A. Somatic hypermutagenesis in immunoglobulin genes. III. Somatic mutations in the chicken light chain locus. *Biochimica et Biophysica Acta* **1306**, 171–178 (1996).
- Rogozin, I. B., Pavlov, Y. I., Bebenek, K., Matsuda, T. & Kunkel, T. A. Somatic mutation hotspots correlate with DNA polymerase ϵ error spectrum. *Nature Immunology* **2**, 530–536 (2001).
- Pham, P., Bransteitter, R., Petruska, J. & Goodman, M. F. Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation. *Nature* **424**, 103–107 (2003).
- KewalRamani, V. N. & Coffin, J. M. Virology. Weapons of mutational destruction. *Science (New York, N.Y.)* **301**, 923–925 (2003).
- Brash, D. E. & Haseltine, W. A. UV-induced mutation hotspots occur at DNA damage hotspots. *Nature* **298**, 189–192 (1982).
- Nik-Zainal, S. *et al.* Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*, **534**, 47–54 (2016).
- Neuberger, M. S., Harris, R. S., Di Noia, J. & Petersen-Mahrt, S. K. Immunity through DNA deamination. *Trends in Biochemical Sciences* **28**, 305–312 (2003).
- Rebhandl, S., Huemer, M., Greil, R. & Geisberger, R. AID/APOBEC deaminases and cancer. *Oncoscience* **2**, 320–333 (2015).
- Bhagwat, A. S. *et al.* Strand-biased cytosine deamination at the replication fork causes cytosine to thymine mutations in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 2176–2181 (2016).
- Bransteitter, R., Pham, P., Calabrese, P. & Goodman, M. F. Biochemical analysis of hypermutational targeting by wild type and mutant activation-induced cytidine deaminase. *The Journal of Biological Chemistry* **279**, 51612–51621 (2004).
- Helico, L., Pham, P., Calabrese, P. & Goodman, M. F. APOBEC3G DNA deaminase acts processively 3' → 5' on single-stranded DNA. *Nature Structural & Molecular Biology* **13**, 392–399 (2006).
- Roberts, S. A. *et al.* Clustered mutations in yeast and in human cancers can arise from damaged long single-strand DNA regions. *Molecular Cell* **46**, 424–435 (2012).
- Harris, R. S., Petersen-Mahrt, S. K. & Neuberger, M. S. RNA editing enzyme APOBEC1 and some of its homologs can act as DNA mutators. *Molecular Cell* **10**, 1247–1253 (2002).
- Lada, A. G. *et al.* AID/APOBEC cytosine deaminase induces genome-wide kataegis. *Biology Direct* **7**, 47 (2012).
- Lada, A. G. *et al.* Mutator effects and mutation signatures of editing deaminases produced in bacteria and yeast. *Biochemistry (Russ. Biokhimiia)* **76**, 131–146 (2011).
- Petersen-Mahrt, S. K., Harris, R. S. & Neuberger, M. S. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* **418**, 99–103 (2002).
- Rogozin, I. B. & Pavlov, Y. I. The cytidine deaminase AID exhibits similar functional properties in yeast and mammals. *Molecular Immunology* **43**, 1481–1484 (2006).
- Taylor, B. J. *et al.* DNA deaminases induce break-associated mutation showers with implication of APOBEC3B and 3A in breast cancer kataegis. *eLife* **2**, e00534 (2013).
- Lu, Z. *et al.* BCL6 breaks occur at different AID sequence motifs in Ig-BCL6 and non-Ig-BCL6 rearrangements. *Blood* **121**, 4551–4554 (2013).
- Matsumoto, Y. *et al.* *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. *Nature Medicine* **13**, 470–476 (2007).
- Nik-Zainal, S. *et al.* Association of a germline copy number polymorphism of APOBEC3A and APOBEC3B with burden of putative APOBEC-dependent mutations in breast cancer. *Nature Genetics* **46**, 487–491 (2014).
- Burns, M. B., Temiz, N. A. & Harris, R. S. Evidence for APOBEC3B mutagenesis in multiple human cancers. *Nature Genetics* **45**, 977–983 (2013).
- Roberts, S. A. *et al.* An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. *Nature Genetics* **45**, 970–976 (2013).
- Smith, K. S. *et al.* Signatures of accelerated somatic evolution in gene promoters in multiple cancer types. *Nucleic Acids Research* **43**, 5307–5317 (2015).
- Chan, K. *et al.* An APOBEC3A hypermutation signature is distinguishable from the signature of background mutagenesis by APOBEC3B in human cancers. *Nature Genetics* **47**, 1067–1072 (2015).

31. Lynch, M. Rate, molecular spectrum, and consequences of human mutation. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 961–968 (2010).
32. Sale, J. E. Translesion DNA synthesis and mutagenesis in eukaryotes. *Cold Spring Harbor Perspectives in Biology* **5**, a012708 (2013).
33. Cooper, D. N. & Youssoufian, H. The CpG dinucleotide and human genetic disease. *Human Genetics* **78**, 151–155 (1988).
34. Santos, F. *et al.* Active demethylation in mouse zygotes involves cytosine deamination and base excision repair. *Epigenetics & Chromatin* **6**, 39 (2013).
35. Zanutti, K. J. & Gearhart, P. J. Antibody diversification caused by disrupted mismatch repair and promiscuous DNA polymerases. *DNA Repair* **38**, 110–116 (2016).
36. Rogozin, I. B. & Diaz, M. Cutting edge: DGYW/WRCH is a better predictor of mutability at G:C bases in Ig hypermutation than the widely accepted RGYW/WRCY motif and probably reflects a two-step activation-induced cytidine deaminase-triggered process. *Journal of Immunology* **172**, 3382–3384 (2004).
37. Morgan, H. D., Dean, W., Coker, H. A., Reik, W. & Petersen-Mahrt, S. K. Activation-induced cytidine deaminase deaminates 5-methylcytosine in DNA and is expressed in pluripotent tissues: implications for epigenetic reprogramming. *The Journal of Biological Chemistry* **279**, 52353–52360 (2004).
38. Wijesinghe, P. & Bhagwat, A. S. Efficient deamination of 5-methylcytosines in DNA by human APOBEC3A, but not by AID or APOBEC3G. *Nucleic Acids Research* **40**, 9206–9217 (2012).
39. Franchini, D. M. & Petersen-Mahrt, S. K. AID and APOBEC deaminases: balancing DNA damage in epigenetics and immunity. *Epigenomics* **6**, 427–443 (2014).
40. Franchini, D. M., Schmitz, K. M. & Petersen-Mahrt, S. K. 5-Methylcytosine DNA demethylation: more than losing a methyl group. *Annual Review of Genetics* **46**, 419–441 (2012).
41. Siritwardena, S. U., Chen, K. & Bhagwat, A. S. Functions and Malfunctions of Mammalian DNA-Cytosine Deaminases. *Chemical Reviews*, e-publ September **1**, doi: 10.1021/acs.chemrev.6b00296 (2016).
42. Kretzmer, H. *et al.* DNA methylome analysis in Burkitt and follicular lymphomas identifies differentially methylated regions linked to somatic mutation and transcriptional control. *Nature Genetics* **47**, 1316–1325, 3 (2015).
43. Lada, A. G. *et al.* Disruption of Transcriptional Coactivator Sub1 Leads to Genome-Wide Re-distribution of Clustered Mutations Induced by APOBEC in Active Yeast Genes. *PLoS genetics* **11**, e1005217 (2015).
44. Taylor, B. J., Wu, Y. L. & Rada, C. Active RNAP pre-initiation sites are highly mutated by cytidine deaminases in yeast, with AID targeting small RNA genes. *eLife* **3**, e03553 (2014).
45. Haradhvala, N. J. *et al.* Mutational Strand Asymmetries in Cancer Genomes Reveal Mechanisms of DNA Damage and Repair. *Cell* **164**, 538–549 (2016).
46. Pfeifer, G. P., Drouin, R., Riggs, A. D. & Holmquist, G. P. *In vivo* mapping of a DNA adduct at nucleotide resolution: detection of pyrimidine (6–4) pyrimidone photoproducts by ligation-mediated polymerase chain reaction. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 1374–1378 (1991).
47. Evelyne Sage, R. D. a. M. R. *From DNA photolesions to Mutations, Skin Cancer and Cell Death*. (The Royal Society of Chemistry, 2005).
48. Yadav, V. K., DeGregori, J. & De, S. The landscape of somatic mutations in protein coding genes in apparently benign human tissues carries signatures of relaxed purifying selection. *Nucleic Acids Research* **44**, 2075–2084 (2016).
49. Ramiro, A. R. & Barreto, V. M. Activation-induced cytidine deaminase and active cytidine demethylation. *Trends in Biochemical Sciences* **40**, 172–181 (2015).
50. Heintel, D. *et al.* High expression of activation-induced cytidine deaminase (AID) mRNA is associated with unmutated IGVH gene status and unfavourable cytogenetic aberrations in patients with chronic lymphocytic leukaemia. *Leukemia* **18**, 756–762 (2004).
51. Green, M. R. *et al.* Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *Proceedings of the National Academy of Sciences of the United States of America* **112**, E1116–1125 (2015).
52. Rogozin, I. B., Babenko, V. N., Milanese, L. & Pavlov, Y. I. Computational analysis of mutation spectra. *Briefings in Bioinformatics* **4**, 210–227 (2003).
53. Rogozin, I. B., Malyarchuk, B. A., Pavlov, Y. I. & Milanese, L. From context-dependence of mutations to molecular mechanisms of mutagenesis. *Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing*, 409–420 (2005).
54. Rogozin, I. B. & Pavlov, Y. I. Theoretical analysis of mutation hotspots and their DNA sequence context specificity. *Mutation Research* **544**, 65–85 (2003).
55. Beale, R. C. *et al.* Comparison of the differential context-dependence of DNA deamination by APOBEC enzymes: correlation with mutation spectra *in vivo*. *Journal of Molecular Biology* **337**, 585–596 (2004).
56. Xiao, Z. *et al.* Known components of the immunoglobulin A:T mutational machinery are intact in Burkitt lymphoma cell lines with G:C bias. *Molecular Immunology* **44**, 2659–2666 (2007).
57. Pavlov, Y. I. *et al.* Correlation of somatic hypermutation specificity and A-T base pair substitution errors by DNA polymerase ϵ during copying of a mouse immunoglobulin kappa light chain transgene. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 9954–9959 (2002).

Acknowledgements

AGL was supported by Buffett Cancer Center Pilot grant and in part by Research Grant of St. Petersburg State University #1.38.426.2015 to YIP. IBR, ARP, AG and EVK are supported by the intramural funds of the US Department of Health and Human Services (to the National Library of Medicine).

Author Contributions

I.B.R., E.V.K. and Y.I.P. conceived the study; I.B.R. designed and performed data analysis; I.B.R., A.G.L., A.G., A.R.P., M.L.G., S.D., G.N., E.V.K. and Y.I.P. analyzed and interpreted the results; I.B.R., E.V.K. and Y.I.P. wrote the manuscript that was edited and approved by all authors.

Additional Information

Supplementary information accompanies this paper at <http://www.nature.com/srep>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Rogozin, I. B. *et al.* Activation induced deaminase mutational signature overlaps with CpG methylation sites in follicular lymphoma and other cancers. *Sci. Rep.* **6**, 38133; doi: 10.1038/srep38133 (2016).

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