Regional Mutational Signature Activities in Cancer Genomes

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Cancer genomes harbor a catalog of somatic mutations. The type and genomic context of these mutations depend on their causes, and allow their attribution to particular mutational signatures. Previous work has shown that mutational signature activities change over the course of tumor development, but investigations of genomic region variability in mutational signatures have been limited. Here, we expand upon this work by constructing regional profiles of mutational signature activities over 2,203 whole genomes across 25 tumor types, using data aggregated by the Pan-Cancer Analysis of Whole Genomes (PCAWG) consortium. We find that 426 genomes from 20 tumor types display at least one change in mutational signature activities (changepoint), and 174 genomes contain a recurrent changepoint shared by three or more genomes of the same tumor type. Twenty-three recurrent changepoint locations are shared by multiple tumor types. Within these regions, the particular signature changes are often consistent across samples of the same type, and some, but not all are characterized by signatures associated with subclonal expansion. The changepoints we found cannot strictly be explained by gene density, mutation density, or cell-of-origin chromatin state. We hypothesize that they reflect a confluence of factors including evolutionary timing of mutational processes, regional differences in somatic mutation rate, large-scale changes in chromatin state that may be tissue type-specific, and changes in chromatin accessibility during subclonal expansion. These results provide insight into the regional effects of DNA damage and repair processes, and may help us localize genomic and epigenomic changes that occur during cancer development.

Mutational signatures | Cancer evolution | Whole genome sequencing
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Introduction

Cancer is a disease that develops over a lifetime through a series of somatic mutations. The vast majority of these mutations are "passenger mutations", which have little effect on fitness. Various mutagenic processes operate in a cancer throughout its development, which in turn determine the nature of the collection of somatic mutations that accumulate. Different mutagenic processes give rise to distinct mutational patterns, called mutational signatures (Alexandrov et al., 2020). In nearly all cancers, multiple mutagenic processes contribute somatic mutations to individual cancer genomes. The relative contributions of these different processes can be quantified by algorithms that assign an activity (or exposure) to each detected mutational signature (Alexandrov et al., 2020). Recent work (Dentro et al., 2021, Gerstung et al., 2020, Rubanova et al., 2020) has revealed that i) these activities vary during cancer development (i.e., tumor progression) in a cancer-type-specific way and ii) most mutational signatures are primarily active only in either early- or late-tumor progression (Dentro et al., 2021, Gerstung et al., 2020) and methods, like TrackSig, have been developed to reconstruct the evolutionary trajectories of mutational signature activities in individual samples (Rubanova et al., 2020, Harrigan et al., 2020). Taken together, this indicates that mutational signature analysis is a compelling tool in our search for understanding of the processes shaping tumor formation and development. In addition to evolutionary timing of signature activity, somatic mutation rate and mutational signatures have been found to be influenced by genomic and epigenomic factors that exert their effects on the scale of several nucleotides up to a megabase. These factors include sequence context, nucleosome positioning, chromatin state, and replication timing (e.g. Gonzalez-Perez et al. (2019), Hodgkinson et al. (2012), Schuster-Böckler and Lehner (2012), Seplyarskiy and Sunyaev (2021), Supek and Lehner (2019), Vöhringer et al. (2021), Yaacov et al. (2021)). For example, late-replicating and heterochromatic regions accumulate more mutations than early-replicating and euchromatic regions (Supek and Lehner, 2019, 2015, Zheng et al., 2014). This results in tumors accumulating mutations in patterns that can be attributed to chromatin state in the cell of origin. In mismatch repair (MMR)-proficient tumors, the rate of CpG>TpG mutations may correlate with replication timing since MMR corrects mispairings due to 5mC deamination more efficiently in early-replicating areas (Gonzalez-Perez et al., 2019, Supek and Lehner, 2019). These genomic features vary on scales of 1 Mb or less, so analyses of their effects on mutational signature activity are normally confined to particular loci. To our knowledge, the relationship between chromosomal location—over the scale of an entire genome—and mutational signature activity has not been thoroughly investigated. If there exists common evolutionary events that dictate the action of mutational processes in cancers of the same type, we can expect to observe consistent changes to mutational signature activity across genomes. To examine the occurrence of mutational signature activity changes across the genome, we analyzed somatic mutations of 2,203 whole genomes across 25 tumor types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) consortium (Campbell et al., 2020). Using an extension of the TrackSig method (Rubanova et al., 2020), we identified ge-

nomic regions that recurrently exhibit mutational signature changes. These regions frequently showed activity changes in signatures known to be associated with evolutionary timing. Some of these regions were shared among several tissue types, while others appeared to be exclusive to specific tissue types.

Methods

We created an extension of the TrackSig algorithm (Rubanova et al., 2020, Harrigan et al., 2020) to variations in mutation signature activity across the genome. TrackSig is designed to detect evolutionary changes in mutational signature activity by segmenting a ranked list of mutations, sorted by inferred cancer cell frequency into subsequences, i.e. segments, of at least 100 mutations with constant mutation signature activity. We extended TrackSig so that the mutations could be sorted instead via chromosomal location and so that it could be run separately across different chromosomes in the same genome. Thus our new method GenomeTrackSig uses the PELT segmentation algorithm (Killick et al., 2012) to identify regions of the genome where signature activities change (changepoints) while using the EM algorithm (Moon, 1996) to fit a multinomial mixture model to estimate signature activities within each segment defined by changepoints. Previous work by Alexandrov et al. (2020) characterized canonical signatures active within the PCAWG data. These serve as the set of reference signatures for our analyses. We fit activity estimates to all signatures which have been previously determined (Alexandrov et al., 2020) to be active in a given tumor type.

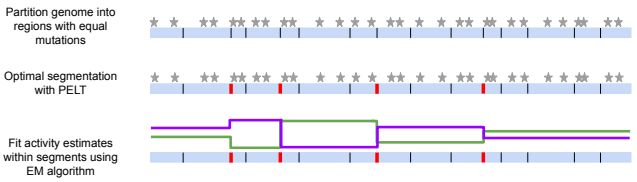

Fig. 1. Overview of GenomeTrackSig algorithm for profiling mutational signature activities across the genome. Stars indicate mutations (top); red bars indicate changepoints (middle); green and purple lines indicate estimated mutation signature activities; y-axis indicates exposure level (bottom). GenomeTrackSig requires at least 100 mutations per segment, fewer mutations are shown here for illustrative purposes.

For efficiency, we partition the set of mutations in a genome into chromosomal regions with equal numbers of mutations, although the number of bases spanned by a region will vary depending on mutation density. We require each segment to include at least one region, so segments have at least one region's worth of mutations. GenomeTrackSig includes flexible options for analyzing samples, either by partitioning and estimating signature activities across an entire genome or on each individual chromosome. The genome-wide approach can capture signature activities that span multiple chromosomes thereby potentially reducing noise in activity estimates at chromosomal ends. On the other hand, the chromosome-wise approach may be more sensitive to activity changes within a chromosome and substantially reduces runtime on samples with many mutations. In general, the chromosome-wise approach is preferred, but we use the genome-wide strategy for samples (and cancers) where chromosomes have less than 100 mutations, as we use this as a minimum segment size to ensure accurate activity estimates.

Results

A. Mutational signature activity is not constant across the genome. We constructed genome-wide signature activity profiles for 2,203 tumors across 25 tumor types (Biliary-AdenoCA, Bladder-TCC, Bone-Osteosarc, Breast-AdenoCA, Cervix, CNS-GBM, Colorect-AdenoCA, Eso-AdenoCA, Head-SCC, Kidney-ChRCC, Kidney-RCC, Liver-HCC, Lung-AdenoCA, Lung-SCC, Lymph-BNHL, Lymph-CLL, Melanoma, Myeloid-MPN, Ovary-AdenoCA, Panc-AdenoCA, Panc-Endocrine, Prost-AdenoCA, Stomach-AdenoCA, Thy-AdenoCA, Uterus-AdenoCA). We found no genomic changepoints in five types of tumor: Liver-HCC (n=326), Ovary-AdenoCA (n=113), Biliary-AdenoCA (n=29), Panc-Endocrine (n=76), and Myeloid-MPN (n=31). A lack of change in signature activity could reflect an even mutational composition across the genome, insufficient number of mutations to characterize a change in signature activity, a lack of large-scale variation in chromatin accessibility, or a lack of substantial changes in mutational composition over tumor development. In the remaining 20 tumor types, at least one sample exhibited changes in signature activities across the genome, indicating that large-scale shifts in signature activities depending on chromosomal location are a common but not ubiquitous phenomenon.

B. Some changepoints are shared across tissue type. We find that changepoint regions are often shared—both across multiple cancers from the same tissue and across multiple tissues. To determine which changepoints within samples from the same tumor type overlap, we fit a kernel density estimate across the vector of genomic locations covered by each changepoint and constructed a possible range for that changepoint location, which contains the changepoint locus identified by GenomeTrackSig, +/- one standard deviation of the density function. We then overlaid all changepoint ranges from that tumor type and counted how many changepoints fell within a sliding window across the genome. Regions with changepoints supported by less than three samples were excluded from consideration. We then deemed a 'recurrent changepoint region' for each tumor type as the center of the region where many samples shared an overlapping changepoint; i.e. the window within a region in which the maximum number of changepoints fell. Of the 20 tumor types that show any changes in signature activity across their genomes, 12 contain recurrent changepoint regions (Fig. 2). Interestingly, the changes in signature activity at these regions are often highly similar across samples, both in direction and magnitude. For example, all 55 melanoma samples that have a changepoint overlapping with the chromosome 1:47,000,000-1:51,000,000 region show a decrease in SBS7b activity and increase in SBS7a activity in the direc-

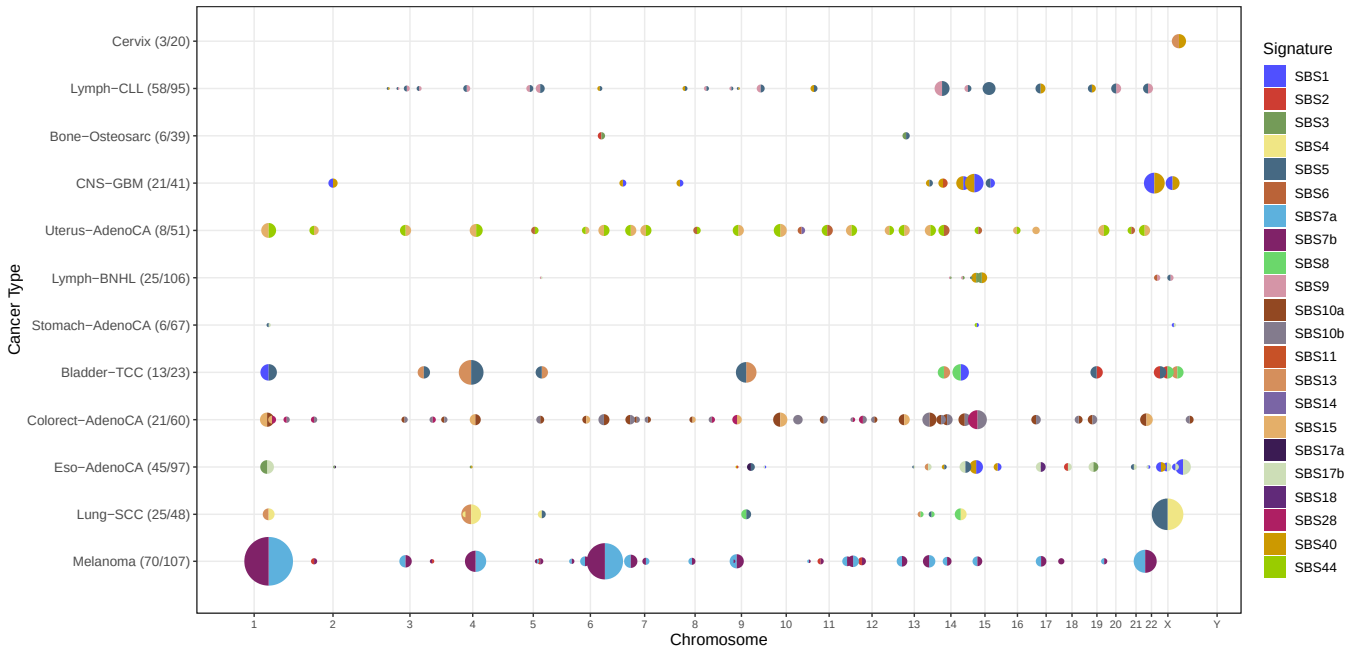

Fig. 2. Recurrent changepoint regions across 12 tumor types. We observe changepoints in 426/2,203 samples. One hundred seventy-four genomic regions are covered by a changepoint found in more than two samples for a given tumor type. Each of these recurrent changepoint regions are indicated by a point, where COSMIC V3 signatures (Alexandrov et al., 2020, Tate et al., 2019) are indicated by color. Changepoints are summarized by the signature whose activity decreases the most on average (left half) and the signature whose activity increases the most on average (right half). Proportion of samples exhibiting a changepoint is encoded by size, and the number of samples affected is indicated in the tumor type label (n/N). Changepoints that appear in fewer than three samples are omitted.

Tumor Type	Samples with at least one changepoint / N total samples	Mean # changepoints per sample	Median # changepoints per sample	Mean changepoint magnitude	Median changepoint magnitude	Top 3 signatures with greatest changes	Clonal signatures	Subclonal signatures
Melanoma	74/107	11	4	0.15	0.13	7b, 7a, 2	7a, 7b	5
Lung-SCC	35/48	9	4	0.05	0.04	4, 13, 8	4	5, 2, 13
Eso-AdenoCA	50/97	5	4	0.09	0.07	17b, 40, 1	17	5, 40
Lung-AdenoCA	5/33	2	2	0.16	0.11	5, 13, 2	4	2, 13
Colorect-AdenoCA	21/60	27	5	0.04	0.03	10b, 15, 10a	1, 44	18, 40
Bladder-TCC	16/23	6	3	0.06	0.05	13, 8, 5	2, 13	5
Stomach-AdenoCA	8/67	3	2	0.13	0.11	17b, 40, 15	1	18
Head-SCC	1/54	2	2	0.15	0.15	40, 4, 5	5	2, 13
Lymph-BNHL	29/106	3	2	0.2	0.15	3, 9, 6	9	17a, 17b, 40
Uterus-AdenoCA	9/51	41	48	0.06	0.05	15, 44, 6	1	2, 13, 40, 44
CNS-GBM	24/41	6	4	0.05	0.03	40, 5, 1	1	40
Kidney-RCC	8/144	1	1	0.08	0.02	5, 40, 13	40	1
Breast-AdenoCA	18/193	3	2.5	0.25	0.2	2, 5, 13	3, 5	2, 3, 13, 18
Panc-AdenoCA	12/238	2	2	0.38	0.34	2, 13, 1	1, 5	2, 3, 13, 17a, 17b, 18, 40
Bone-Osteosarc	13/39	3	2	0.31	0.35	2, 5, 3	2, 13	40
Prost-AdenoCA	11/145	2	2	0.17	0.16	13, 5, 40	5	40
Lymph-CLL	66/95	3	3	0.22	0.19	5, 40, 9	9	5
Kidney-ChRCC	5/38	4	4	0.24	0.22	2, 5, 40	5	1, 2, 13, 40
Cervix	13/20	2	2	0.08	0.07	40, 13, 5	1, 2, 13, 5	40
Thy-AdenoCA	8/29	2	1	0.2	0.15	5, 40, 1	5	2, 13

Table 1. Overview of changepoints discovered across 20 cancer types. Magnitude of each changepoint is measured as the cosine distance of the signature activity vector on either side of the changepoint. For each cancer type containing changepoints, the three signatures with the greatest absolute value of activity changes across all changepoints in that cancer are listed. Signatures are indicated as "clonal" or "subclonal" respectively based on where their activity is highest, as described by Dentro et al. (2021).

tion of the numbering (Fig. 3). In Uterus-AdenoCA samples, the signatures which changed most at every recurrent changepoint are associated with defective DNA mismatch repair, most consistently SBS15 and SBS44 (Fig. 2). In other cancers, the most affected signatures are highly variable from changepoint-to-changepoint: seven different signatures are represented among the most substantial activity changes at recurrent changepoints in Eso-AdenoCA samples, including SBS1, SBS2, SBS3, SBS5, SBS17a, SBS17b, and SBS40 (Fig. 2). We also find that 23 recurrent changepoints are shared by multiple tumor types. The most common recurrent changepoint region is 1:47,000,000-1:51,000,000, which appears in Melanoma, Uterus-AdenoCA, Lung-SCC, Bladder-TCC, and Stomach-AdenoCA (Fig. 2). Other recurrent changepoint regions shared by three tumor types include coordinates 1:190,000,000-1:194,000,000 (Colorect-AdenoCA, Melanoma, Uterus-AdenoCA), 3:194,000,000-3:198,000,000 (Bladder-TCC, Eso-AdenoCA, Lung-SCC),

5:22,000,000-5:26,000,000 (Colorect-AdenoCA, Lymph-CLL, Melanoma), and 13:94,000,000-13:98,000,000 (Bladder-TCC, Lung-SCC, and Uterus-AdenoCA) (Fig. 2). Despite the consistency of changepoint locations across multiple samples and tissues, it is unlikely that all these changepoints result from mutations in specific genes near the changepoint region. For one, changepoint regions span multiple megabases, so it is difficult to attribute the changepoint to mutations in any one particular gene. That said, some changepoint regions contain genes that may play a role in tumorigenesis such as Lymph-CLL changepoint regions encompassing CBL proto-oncogene B and NRP2 (Goel et al., 2012, Liyasova et al., 2015), and a changepoint region shared by Lung-SCC and Bladder-TCC which encompasses the DDX17 gene (Wu, 2020). However, we were unable to detect any clear pattern of gene function at changepoint regions.

C. Many changepoints are characterized by signatures associated with subclonal expansion. In certain cancers, we find that recurrent changepoints show a shift in activity from signatures associated with early cancer development to signatures associated with subclonal expansion. For instance, at 13/21 recurrent changepoints in Lymph-CLL samples, the most dramatic signature activity changes were increases in SBS9 and decreases in SBS5, or vice versa. We validated that these changepoints indeed reflect a shift between early and late signatures by constructing evolutionary trajectories for 90 Lymph-CLL samples using TrackSig (Rubanova et al., 2020). Figure 4 shows the distribution of activity changes from early to late development across all samples, in which we observe a definitive decrease in SBS9, and increase in SBS5 activity. In other cancers, signatures which account for the most dramatic activity changes at recurrent changepoint regions do not show a clear timing association. For example, SBS7a and SBS7b change most dramatically across the genome in melanoma samples (Fig. 2), yet show a weak association with evolutionary timing (from early-occurring to late-occuring mutations, SBS7a activity decreases by 8.7% +/- 12.2% on average and SBS7b activity increases by 1.7% +/- 9.1% on average).

Chronic lymphocytic leukemias (Lymph-CLL) tend to exhibit high SBS9 activity early in development, and show decreasing SBS9 and increasing SBS5 and SBS40 activities as subclones form (Fig. 4; Dentro et al. (2021)). SBS9 activity is attributed to mutagenesis induced via DNA polymerase eta. These mutations can occur in healthy lymphoid cells as part of a somatic hypermutation process, which introduces mutations to antibody-coding sequence in order to generate sequence variability and produce antibodies with higher specificity (Seki et al., 2005). SBS9 activity is elevated in Lymph-CLL samples that possess immunoglobulin gene hypermutation (Alexandrov et al., 2020, Gerstung et al., 2020), a mutational process that typically occurs early in tumor development (Dentro et al., 2021, Seifert et al., 2012). Interestingly, changes in signature activity were distributed at many loci in Lymph-CLL samples, whereas in B-cell non-Hodgkin lymphoma (Lymph-BNHL), which also undergoes polymerase eta dependent somatic hypermutation, changes in signature activity were concentrated on chromosome 14, close to the immunoglobulin gene hypermutation (IGH) locus. While the etiologies of SBS5 and SBS40 are unknown, their activity correlates with patient age, and SBS5 has been associated with proliferation (Alexandrov et al., 2020, Franco et al., 2019).

Lymph-CLL signature activity has previously been seen to be associated with evolutionary timing. Similar cancers also evolve in consistent patterns (Dentro et al., 2021, Rubanova et al., 2020, Campbell et al., 2020). In keeping with this, we observe recurrent changepoints that may be indicative of important evolutionary (and shared) changes that occur over the progression of these tumors, such as timing-dependent changes in chromatin state at these regions. Regional mutation rates and mutational signatures are influenced by chromatin state, and under normal cell growth conditions we would expect to see higher mutation rates in heterochromatic than euchromatic regions (Vöhringer et al., 2021, Yaacov et al., 2021). However, the compounding DNA damage and dysfunction in DNA repair that occurs as cancers develop could induce changes in chromatin state, making regions vulnerable to mutations late in development that were not vulnerable during early cancer formation. Ergo, if the chromatin state at a particular region changes during tumor evolution, this may also manifest as a regional change in signature activities.

D. Changepoints are not associated with any single phenomenon. We examined the correlation between changepoint locations and changes in gene density, mutation density, and when available, tissue-specific measures of chromatin accessibility (Polak et al., 2015) to investigate what underlying genomic or epigenomic features could account for the observed distribution of changepoints. Given that tissue-specific or cancer-specific DNAse-Seq or ATAC-Seq data is not available for all tumor types in our dataset, we cannot draw broad conclusions about the relationship between local changes in chromatin accessibility and changepoint placement. However, it is interesting to note that most melanoma samples display low correlation between DNAse-I accessibility index and changepoint occurrence (mean correlation = 0.12), thus it does not seem that local chromatin state changes alone can explain changes in signature activity (Fig. 5A-B). Changes in gene density or mutation density alone also fail to explain the distribution of observed changepoints. For instance, the mean correlations between changepoint occurrence and gene and mutation density across 74 melanoma samples are 0.13 and 0.11, respectively (Fig. 5C-D). Changepoints occur in both gene and mutation-dense and gene and mutation-poor regions, changes in both variables are often unaccompanied by changepoints, and we could observe no obvious biased placement of changepoint with respect to these variables. Furthermore, recurrent changepoints occur across multiple samples and cancer types, where these features vary.

To further investigate if changes in mutation density affect changepoint placement, we also analyzed the distances be-

Fig. 3. Similar signature changes at the 1:47,000,000-1:51,000,000 region in 55/107 melanoma samples. Each polar coordinate chart shows the changes in mutational signature activity in a melanoma sample for a single recurrent changepoint region, at 1:47,000,000-1:51,000,000. Signatures on the left half of the chart decrease in activity at the changepoint and signatures on the right half increase in activity at the changepoint. The height of each slice denotes the numerical change in signature activity. The width of the slice indicates how many signatures increased or decreased at 1:47,000,000-1:51,000,000 in that sample (wider slice = few signatures increasing, or vice versa).

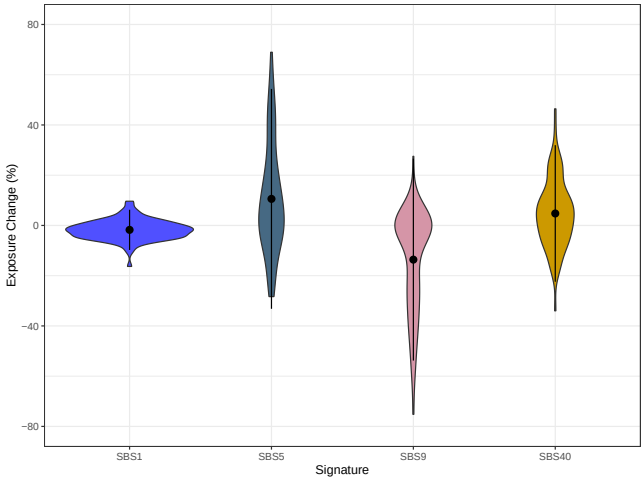

Fig. 4. Activity change in four SBS signatures during cancer evolution in 90 Lymph-CLLs. Violin plots showing the distribution of signature activity changes (y-axis) from early to late stages of cancer development constructed using TrackSig (Rubanova et al., 2020, Harrigan et al., 2020, **?**) for 90 chronic lymphocytic leukemia samples. Dot indicates means, vertical line spans +/- one standard deviation.

tween changepoints and copy number aberrations (CNAs), and the distances between changepoints and kataegis events. We conducted a randomization test to determine, for each sample, if a significant proportion of the changepoints in the sample overlap with a CNA. We found that overall, 60/426 samples with changepoints have a higher proportion of changepoints overlapping with a CNA than would be expected at random. Cervical cancers have the highest degree of changepoint/CNA co-occurrence, with 7 of 13 samples with changepoints displaying significant overlap. No Lymph-CLL, Prost-AdenoCA, Stomach-AdenoCA, or Colorect-AdenoCA samples showed significant changepoint/CNA overlap. These results emphasize that the changepoints detected by TrackSig reflect genuine shifts in mutational composition, not just mutation density. Further, we find that structural variation in the genome cannot explain the observed distribution of mutational signature activity changes.

Across the 2044/3059 changepoints within samples containing a kataegis event (Dentro et al., 2021, Nik-Zainal et al., 2012), these events were often distantly separated. The mean and median distances of changepoints to the nearest kataegis event were 174 Mb and 10 Mb, respectively, although some are relatively close: 634/2044 changepoints were located within 1 Mb from a kataegis event, suggesting that kataegis is one potential cause of a changepoint but not the majority cause. We observed a similar pattern when analyzing recurrent changepoints. The mean and median distances to a kataegis event among recurrent changepoints were 173 Mb and 4 Mb respectively, and 735/1159 changepoints were located >1 Mb away from their nearest kataegis event. One reason for the partial association of kataegis and changepoints may be that the vast majority of kataegis events involve APOBEC deaminases, which are represented by SBS2 and SBS13 (Alexandrov et al., 2020, Nik-Zainal et al., 2012). In this case, the hypermutation would result in a significant change in the local activity of these signatures, which would be detected by GenomeTrackSig. The localized hypermuta-

tion of the IGH locus in Lymph-BNHL may result in a SBS9-enriched kataegic event, which may explain both the high incidence of kataegis in Lymph-BNHL as well as the proximity of kataegis to the recurrent changepoints in that cancer (mean and median distances = 7 Mb and 6 Mb, respectively).

Lymph-BNHL exhibits at least one polymerase eta-driven hypermutation hotspot on every chromosome (Campbell et al., 2020), however changepoints were almost exclusively found on chromosome 14 (Fig. 2). In contrast, Lymph-CLL contains many recurrent change points across different chromosomes (Fig. 2) but relatively few kataegis foci (Campbell et al., 2020). Although these two cancers have commonalities in their activities of SBS1, SBS9, SBS5, and SBS40 (Alexandrov et al., 2020) as well as being the only two cancer types known to display polymerase eta-driven kataegis (Campbell et al., 2020), within them we observe nearly opposite relationships between GenomeTrackSig changepoints and kataegis foci. In summary, kataegis has some association with GenomeTrackSig changepoints but it does not explain the majority of changepoints and its presence does not always give rise to a changepoint.

Discussion

Here we have introduced a new method, GenomeTrackSig, to detect region-specific changes in mutational signature activity within cancer genomes. Using this method we have demonstrated frequent changes in mutational signature activities over large chromosomal domains in a variety of cancers. We have also found a surprising number of recurrent changepoints in signature activities shared across cancers of the same type and among cancers of different types.

The scale, >1 Mb, at which GenomeTrackSig can generally detect changes in mutational signature activity is much larger than regional factors already known to influence mutation rates, which makes it surprising that we see so many changepoints. For example, regional mutation rate varies due to changes in replication timing, differential activity of DNA repair mechanisms, and chromatin accessibility (Schuster-Böckler and Lehner, 2012, Supek and Lehner, 2019, Vöhringer et al., 2021, Yaacov et al., 2021, Supek and Lehner, 2015, Zheng et al., 2014). In samples with many mutations, in which a changepoint can demarcate smaller regions (< 1 Mb in size), we may be able to attribute changes in signature activities to some of these factors that cause regional differences in the somatic mutation rate, especially if these factors change substantially over tumor development (see below). However, our effort to quantify this phenomenon using readily-measured genomic features – gene density, mutation density, structural and copy number variants, or kataegis (Fig. 5) – established that the signature changes we are detecting reflect a genuine shift in underlying mutational distributions and cannot be fully explained by these local, smaller-scale factors alone (Fig. 5).

Perhaps, instead our changepoints demarcate large-scale chromosomal organization, with each large domain having its own underlying mutational distribution driven by domain-specific DNA damage and repair dynamics. Given that signature changes at recurrent changepoints are often consistent within a tissue yet variable across tissues, it is possible that such chromosomal domains can occur at similar locations in multiple tissue types yet exert tissue-specific effects on the mutational landscape. One intriguing possibility is that these large-scale domains are delineated by the 3D organization of the cancer genome – representing, for example, larger topologically associating domains (TADs) Yu and Ren (2017).

The signature dynamics at the most common changepoint in melanoma samples support our hypothesis that changepoints are driven by chromatin state variation over large scales. The changepoint region at 1:47,000,000-51,000,000 is found in 77 samples from 5 tumor types, 55 of those melanomas. As shown in Fig. 4, all 55 melanomas display strikingly consistent decreases in SBS7b activity and increases in SBS7a activity at this changepoint. An analysis of the genomic properties influencing mutational signature activities by Vöhringer and colleagues (2021), using a de novo signature extraction method, identified two signatures exclusively occurring in skin cancers which highly resemble SBS7a and SBS7b in terms of their mutational distributions and associations with UV exposure. Their study found that the SBS7a-like signature has high activity in quiescent chromatin, whereas SBS7b-like is enriched in active chromatin and has a strong transcriptional strand bias. They suggested this differential signature activity across chromatin states reflects different operative DNA repair mechanisms. In this scenario, SBS7a-like activity reflects UV damage cleared by global genome nucleotide excision repair (GG-NER), which operates in quiescent and active regions, and SBS7b-like activity reflects damage cleared by a combination of GG-NER and transcription-coupled nucleotide excision repair (TC-NER), the latter of which operates in open chromatin and is activated by template strand lesions on actively transcribed genes (Vöhringer et al., 2021). Therefore, the activity shifts between SBS7a and SBS7b that we observe at recurrent changepoint regions in melanomas may reflect large-scale changes in chromatin state, coupled with changes in active DNA repair processes.

Based on signature activity profiles from Lymph-CLL samples, we also hypothesize that large-scale changes in chromatin state sometimes occur in a timing-dependent manner. We observe changepoints in which early-development signatures decrease in activity and late-development signatures increase in activity, and vice versa. Cancer development is marked by a redistribution of the mutational landscape, as exposures to mutagens and DNA repair failures strip active genes in euchromatic regions of the preferential repair that they normally receive (Supek and Lehner 2019). Thus, we may see higher activity of late-development signatures in regions that normally have a low mutation rate, but which become vulnerable to mutation during subclonal expansion when different mutational processes are active than were early on. Conversely, late-replicating regions of closed chromatin might display higher activity of early-development signatures since these tend to have higher mutation rates under normal conditions.

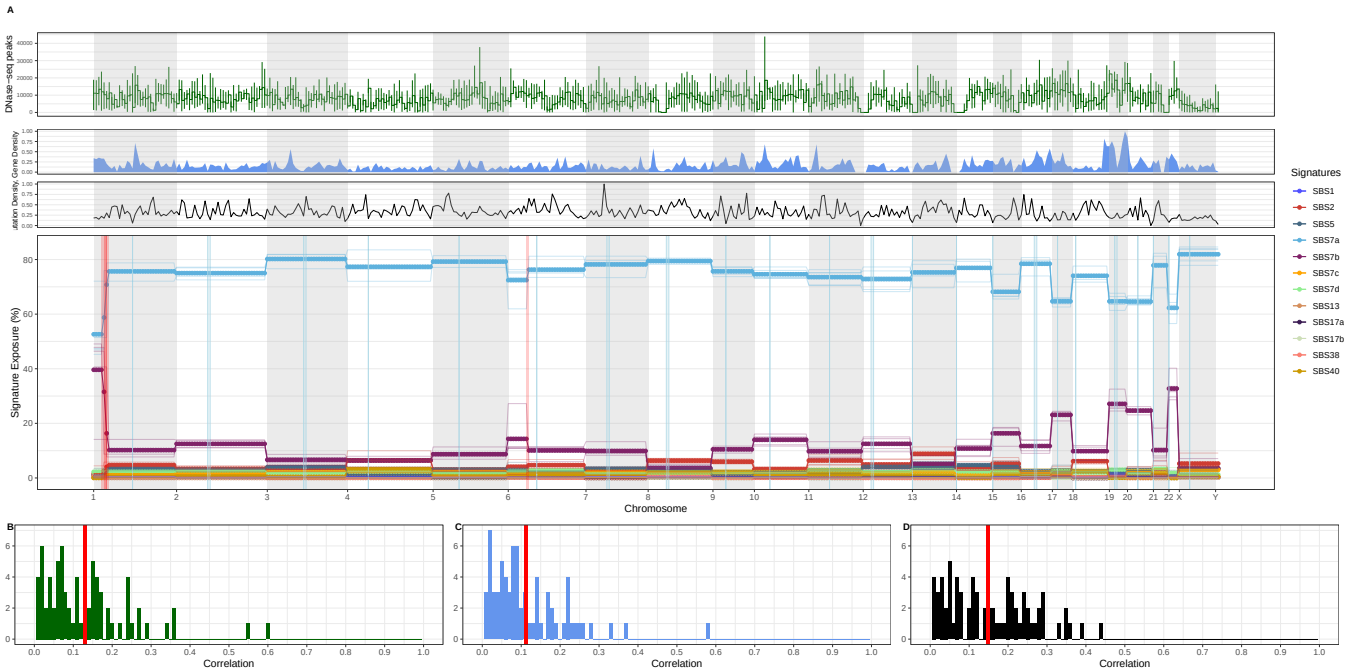

Fig. 5. The association of signature activity profiles and chromatin accessibility, gene density, and mutation density profiles. A: Signature activity profile (chromosome-wise) for a representative melanoma sample with 94,000 mutations and four changepoints. Colored lines denote signature activities across bins of 200 mutations. Alternating gray and white bars denote chromosomal boundaries, and vertical blue lines show centromere positions. Red vertical lines show changepoint locations, and the opacity of these lines denotes confidence in that changepoint's location. Above, black line plot depicts mutation density at each bin across the genome. Mutation density is normalized such that the bin with the highest density throughout the genome is scaled to one and the bin with the minimum density is scaled to zero. Above, blue area plot represents the average gene density at each bin, as determined from gene counts in hg19. Gene density is normalized in the same manner as mutation density. Above, green line plot depicts chromatin accessibility throughout a primary melanocyte genome as determined via DNAse-I accessibility index (Polak et al., 2015). Horizontal lines at each bin show the average chromatin accessibility across each bin, and vertical lines depict the range of chromatin accessibility values within each bin. B, C, and D show the distribution of correlation values between changepoint locations and DNAse-I accessibility index, gene density, and mutation density, respectively, across the 74 melanoma samples which contain changepoints. Mean correlation is highlighted in red on each plot.

Via mutational signature analysis, we have identified that mutational signature activities change over chromosomal domains. These changes can be highly consistent on multiple levels. We hypothesize that, among other factors, our method is detecting the impacts of large-scale changes in chromatin state. Recurrent changepoints in melanoma samples provide compelling evidence that regions on either side of a changepoint differ in terms of their chromatin accessibility and DNA repair dynamics. Furthermore, changepoints that appear in multiple tissue types raise the possibility that such wide-scale epigenomic changes are common events in the course of cancer development. These results call for further exploration into how factors like chromatin state and DNA repair mechanisms vary over wider domains, and which elements of these changes are characteristic of many cancers and which are tissue-specific. Interestingly, changepoints sometimes reflect a shift in activity between signatures characteristic of early tumor development and signatures characteristic of subclonal expansion. Thus, we hypothesize that large-scale changes in chromatin accessibility may also occur in a timing-dependent manner, and can demarcate regions that are more likely to be impacted by mutations early or late in a cancer's evolutionary trajectory. This finding may be clinically relevant given that intra-tumor heterogeneity is a mechanism of therapeutic resistance and therefore presents significant challenges for treatment (Maley et al., 2006, McGranahan and Swanton, 2017, Mroz et al., 2013). Therefore, genome-wide mutational signature analysis can help us further characterize and localize the genomic and epigenomic changes that occur during tumor development.

ACKNOWLEDGEMENTS

QM is a CCAI chair and is partially supported by a NIH/NCI Cancer Center Support Grant P30 CA008748

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