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'PEAR-ing' Genomic and Epigenomic Analyses for Cancer Gene Discovery

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Summary

Somatic structural variants in tumor genomes can deregulate transcription through repositioning of enhancer elements. A new method, PEAR-ChIP, leverages paired-end H3K27ac ChIP-seq and current current computational methods to identify such events.

Cancer genomes often contain recurrent somatic structural variations (SVs) in the form of DNA amplifications, deletions, inversions, and translocations. Conventional approaches to pinpointing oncogenic drivers and tumor suppressor regions within SVs usually focused on putative oncogenes or tumor suppressor genes found within minimal common regions of alteration (1). This method has proven to be widely successful, leading to the detection of novel and recurrent oncogenes and tumor suppressor genes across several cancer types (2). In the case of DNA translocations, a similar approach has been used, where mapping of recurrent DNA breakpoints have revealed oncogenic gene fusion products that generate chimeric oncoproteins. These strategies prioritized candidate genes based on the rationale that the SVs should correspond with gene-level associated alteration such as increased DNA copy number or coding sequence change. However, by applying a gene-centric focus, SVs targeting non-coding regions of the genome have been largely unexplored.

Unclear in cancer genomic landscapes is the prevalence of SVs that lead to gene activation, independent of gene-disruption, such as rearrangement of DNA regulatory elements in noncoding regions of the genome. Identifying such events has been perhaps challenging in the past due to a limited capacity to detect complex rearrangements at high-resolution, and the ability to ascribe function to these alterations by determining the precise genes they regulate. These difficulties have been overcome, at least in part, by the increasing feasibility

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of whole-genome sequencing, allowing for more thorough characterization of cancer genomes. Furthermore, advancements in chromatin mapping using techniques such as chromatin immunoprecipitation combined with high-throughput sequencing (ChIP-seq) have unraveled the regulatory landscape of both normal and cancer epigenomes (3, 4). Histone modifications, specifically, indicate functional regions of the genome such as enhancers, marked by histone H3 lysine 4 mono-methylation (H3K4me1), and the potential activation status of those enhancers marked by histone H3 lysine 27 acetylation (H3K27ac).

Two recent studies leverage and integrate these genomic and epigenomic technologies, to identify highly recurrent SVs that reposition distal enhancer elements proximal to genetically intact oncogenes, termed ‘Enhancer Hijacking’ or ‘Enhancer Hitchhiking’ (5, 6). In aggressive subgroups of Medulloblastoma (Group 3 and 4), various classes of SVs such as tandem duplications, deletions, inversions, translocations, and other more complex rearrangements, converge to activate *GFI1* or *GFI1B* oncogenic expression. This is accomplished by repositioning the intact *GFI1B* or *GFI1B* genes in close proximity with distal super enhancers (Highly active enhancer regions marked by extensive H3K27 acetylation) (6). Importantly, *GFI1* and *GFIB* activation through ‘Enhancer Hijacking’, have not been reported in other cancers, and are the most prevalent driver events in Group 3 medulloblastoma. Similar observations have been observed in a type of acute myeloid leukemia (AML), characterized by chromosome 3q rearrangements (inv(3)/t(3;3)) that lead to aberrant expression of the stem-cell regulator *EVI1* (5). The mechanism of *EVI1* activation is caused by a chromosomal translocation, which relocates a *GATA2* super enhancer proximal to the *EVI1* oncogene. This single SV event not only activates *EVI1* expression, but also removes an enhancer regulating *GATA2*, thereby leading to mono-allelic *GATA2* expression and haplo-insufficiency. Examples in medulloblastoma and AML suggest that ‘Enhancer Hijacking’ events may be potentially common and driver alterations in other cancer types, and underscores the need for combined methodologies that leverage information from both genomic and epigenomic platforms.

In the current issue, Ryan and Drier et al., (2015) present a novel approach called PEAR-ChIP, which integrates H3K27ac ChIP-seq with paired-end sequencing (7) (Figure 1). Utilizing pre-existing computational tools to detect genomic rearrangements, PEAR-ChIP maps structural variations involving acetylated regulatory elements (PEAR-ChIP, Pinpointing Enhancer-Associated Rearrangements by Chromatin Immunoprecipitation and Paired-End Sequencing). They apply this methodology to investigate a cohort of 14 primary patient biopsies and 8 cell line models representing a diversity of B-cell lymphomas. Importantly, the authors validated several known SVs, and identified numerous types of novel chromosomal rearrangements that delineate various B-cell lymphoma subtypes.

This approach is first validated in mantle cell lymphoma (MCL) primary tissue and cell lines, all of which harbor reciprocal translocations between the *IGH* J recombination region (Chromosome 14) and a gene-desert region located > 300 kb from *CCND1* (Chromosome 11). In all samples, H3K27ac enrichment was observed, and extended from the *IGH* μ intronic enhancer and overlapping the J recombination region. PEAR-ChIP identified the precise t(11;14) breakpoints, all of which contained peaks of acetylation signal. This methodology was then applied to a heterogeneous cohort of primary high-grade B-cell

lymphomas (HGBs), cell lines, and lymph node biopsies from patients with chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL). PEAR-ChIP identified several known rearrangements linking an *IGH* enhancer exclusively to the 5' side of *MYC* revealing potentially significant and specific mechanisms of cis-regulation. This was in contrast to a collection of non-IGH rearrangement breakpoints that occurred on the 3' side of *MYC*.

In cases where HGB cell lines were also profiled by whole-genome sequencing, PEAR-ChIP detected all six inter-chromosomal or large-scale inversions with improved sensitivity (2-17 times more supporting reads) despite lower sequencing coverage (10-31 fold lower). Further, PEAR-ChIP was sensitive for the detection of other large-scale rearrangements containing several known translocation targets, including *BCL2*, *CIITA*, and *PDCD1LG2*, and novel translocation partners including *PDCD1LG2-NCOA3* and *CIITA-IL4R*. In addition to translocation events, PEAR-ChIP identified several other classes of structural variations that repositioned candidate enhancer elements of oncogenic significance, including small-scale intra-chromosomal deletions and inversions. In several examples, kilobase-scale tandem duplications were detected targeting acetylated putative enhancers upstream of the rho GTPase-activating gene *TAGAP*, and duplication of regions harboring an interferon-responsive enhancer upstream of the inducible nitric oxide synthase gene *NOS2*. These events represent potentially novel mechanisms of oncogenic regulation currently unexplored in HGB biology.

Guided by information gained from chromosome conformation capture (3C) experiments, the authors utilized the PEAR-ChIP approach to investigate the complex dynamics of *BCL6* and *MYC* oncogenic regulation in HGB. 3C studies revealed that MCL and SLL were delineated by 5' interacting enhancers with the *MYC* promoter, while HGBs were distinctly characterized by 3' enhancers. In the case of *BCL6*, a gene known to frequently rearrange in HGB, multiple 3' super enhancers were detected and demonstrated to form contact loops with the *BCL6* promoter. Furthermore, PEAR-ChIP was successful in detecting a tandem duplication spanning one of the super enhancers, in addition to breakpoints within acetylated regions proximal to *BCL6*. Understanding the native enhancer-promoter regulation of *BCL6* and *MYC* in HGB, allowed the authors to explore the product of translocations between the *BCL6* and *MYC* loci. In one sample, harboring a t(3;8)(q27;q24) rearrangement, PEAR-ChIP identified a breakpoint that swapped the *BCL6* super enhancer region described above, with the 3' *MYC* enhancer regions prevalent in HGB lines, classified as an 'Enhancer Swap'. The authors report that, in independent rearrangements, the *BCL6* locus is capable of acting as a 'donor' or 'recipient' of enhancer elements, thus revealing novel mechanisms in cancer gene de-regulation.

These findings emphasize that rearrangement of enhancer elements by structural variation are potentially a general mechanism of oncogenic activation across cancer. PEAR-ChIP represents both a sensitive and cost effective approach to identify such alterations. One important consideration is the requirement for breakpoints to be located within acetylated regions in order to be detected by PEAR-ChIP. Breakpoints occurred within acetylated loci in many of the B-cell lymphomas in this study, and Group 3 and 4 Medulloblastomas, but this may not be the case in other cancers. The authors suggest that in such scenarios, one

complementary approach would be to pair the high-resolution breakpoint detection of PEAR-ChIP with a low-resolution, genome-wide platform, such as long-insert mate pair sequencing. While 'Enhancer Hijacking' events represent novel modes of oncogenic regulation that are independent of gene disruption, care should be taken to validate that the effects upon gene expression are indeed direct mechanisms. As shown in this study, and *EVI1* re-arranged AML (5), chromosome conformation capture experiments can complement such analyses providing insights about enhancers in their native loci, and the consequence of their repositioning. Beyond structural variations, recurrent somatic mutations have recently been identified in regulatory regions across different cancer types (8-10). With increasing understanding of cancer epigenomes, armed with integrative tools such as PEAR-ChIP, revisiting cancer genome studies may be warranted, and may reveal novel mechanisms of oncogenic regulation.

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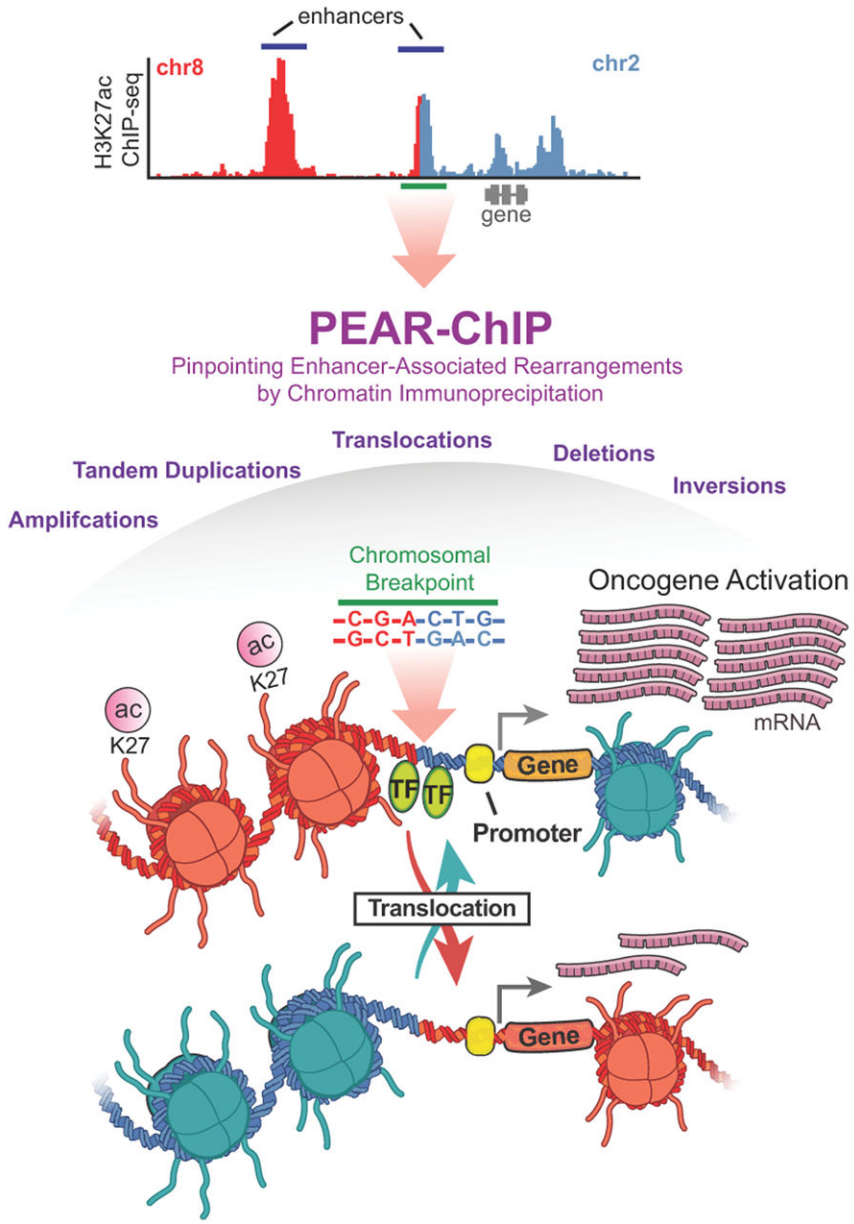

Figure 1.

An illustration of the PEAR-ChIP approach used to identify genomic rearrangements within regions of H3K27 acetylation. The top panel is a representative H3K27ac ChIP-seq profile with chromosome 8 reads in red, and chromosome 2 in blue. The breakpoint region is shown as a green bar, and this local region is expanded in a schematic in the lower panel.