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REVIEW

3D genome and its disorganization in diseases

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Abstract The chromosomes in eukaryotic cells are highly folded and organized to form dynamic three-dimensional (3D) structures. In recent years, many technologies including chromosome conformation capture (3C) and 3C-based technologies (Hi-C, ChIA-PET) have been developed to investigate the 3D structure of chromosomes. These technologies are enabling research on how gene regulatory events are affected by the 3D genome structure, which is increasingly implicated in the regulation of gene expression and cellular functions. Importantly, many diseases are associated with genetic variations, most of which are located in non-coding regions. However, it is difficult to determine the mechanisms by which these variations lead to diseases. With 3D genome technologies, we can now better determine the consequences of non-coding genome alterations via their impact on chromatin interactions and structures in cancer and other diseases. In this review, we introduce the various 3D genome technologies, with a focus on their application to cancer and disease research, as well as future developments to extend their utility.

Keywords 3D genome · Hi-C · Cancer · Disease

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Chromosome conformation capture technologies

The emergence of chromosome conformation capture (3C) technologies is critical for studying chromatin spatial organization. The 3C and 3C-based technological breakthroughs now permit researchers to analyze the three-dimensional (3D) architecture of genome organization in more detail. Here, we review key methodological advances (Table 1) for capturing and mapping complex chromatin interaction landscapes.

The 3C assay (Dekker et al. 2002) was invented by Dekker and colleagues in 2002. It allows us to capture long-range chromatin interaction events between two specific genomic sites. The samples for 3C analysis undergo a series of experimental steps (Fig. 1a), including formaldehyde fixation for in situ cross-linking, digestion with a restriction enzyme, ligation of spatially close DNA ends, and finally detection by polymerase chain reaction (PCR) using primers of the two interested chromatin loci. However, the 3C assay has the limitation that it can only be used for chromatin interaction analysis between two predefined loci. In 2006, a modified technique based on 3C was developed, which is named "chromosome conformation capture on a

Table 1 Overview of chromosome conformation capture technologies (3C) and 3C-based technologies

Chromosome conformation capture technologies		
Assay	Full assay name	References
1 versus 1		
3C	Chromosome conformation capture	Dekker.et al.Science, 2002
1 versus many		
4C	Chromosome conformation capture-on-chip	Simonis.et al.Nat Genet, 2006
4C-seq	Chromosome conformation capture-on-chip combined with high-throughput sequencing	Van de Werken.et al. Nat Methods, 2012
Many versus many		
5C	Chromosome conformation capture carbon copy	Dostie. et al.Genome research, 2006
Many versus many + protein specific		
ChIA-PET	Chromatin interaction analysis paired-end tag sequencing	Fullwood. et al. Nature, 2009
Many versus all		
Capture-C	Chromosome conformation capture coupled with oligonucleotide capture technology	Hughes. et al. Nat Genet, 2014
Capture-HiC	Hi-C coupled with oligonucleotide capture technology	Borbala Mifsud. et al. Nat Genet, 2015
Many versus all + antibody to recapture		
PLAC-seq	Proximity ligation-assisted ChIP-seq	Fang. et al.Cell Res, 2016
HiChIP	Protein-centric chromatin conformation assay	Mumbach. et al.Nat Methods, 2016
All versus all		
Hi-C	Genome-wide chromosome comformation capture	Lieberman-Aiden. et al.Science, 2009
In situ Hi-C	Genome-wide chromosome comformation capture with in situ ligation	Rao. et al. Cell, 2014
DNase Hi-C	Genome-wide chromosome comformation capture with Dnase I digestion	Ma, W. et al. Nat Methods, 2015
Single-cell Hi-C	Single-cell genome-wide chromosome comformation capture	Nagano. et al. Nature, 2013 Ramani. et al. Nat Methods, 2017 Stevens. et al. Nature 2017
Micro-C/MNase-C/MACC	Micrococcal nuclease (MNase)	Hsieh. et al. Cell, 2015 Mieczkowski. et al. Nature communications, 2016
A nuclease hypersensitivity assay: open chromatin detection		
ATAC-seq	Transposase-accessible chromatin using sequencing	Buenrostro. et al. Nat Methods, 2013
ATAC-see	Transposase-accessible chromatin with visualization	Chen, X. et al. Nat Methods, 2016
DNase Hi-C	3D structure of open chromatin architecture	Ma, W., et al. Nat Methods, 2015
DNase-seq	A high-resolution technique for mapping active gene regulatory elements across the genome	Song. et al. Cold Spring Harbor protocols, 2010

chip" (4C) (Simonis et al. 2006; Van de Werken et al. 2012). 4C has made it possible to detect chromatin interactions between one locus and multiple loci. To establish a massively parallel solution for capturing multiple-to-multiple loci interactions, a high-throughput 3C approach has been developed and named "3C-Carbon Copy" (5C) (Dostie et al. 2006), which is used to maps cis- and trans-chromatin interaction networks at the beta-globin locus.

To achieve high-throughput chromatin interaction analysis, in 2009, Dekker and colleagues developed Hi-C, a method combining DNA digestion and ligation with massive parallel sequencing to map the spatial proximity of chromatin at the level of the entire genome

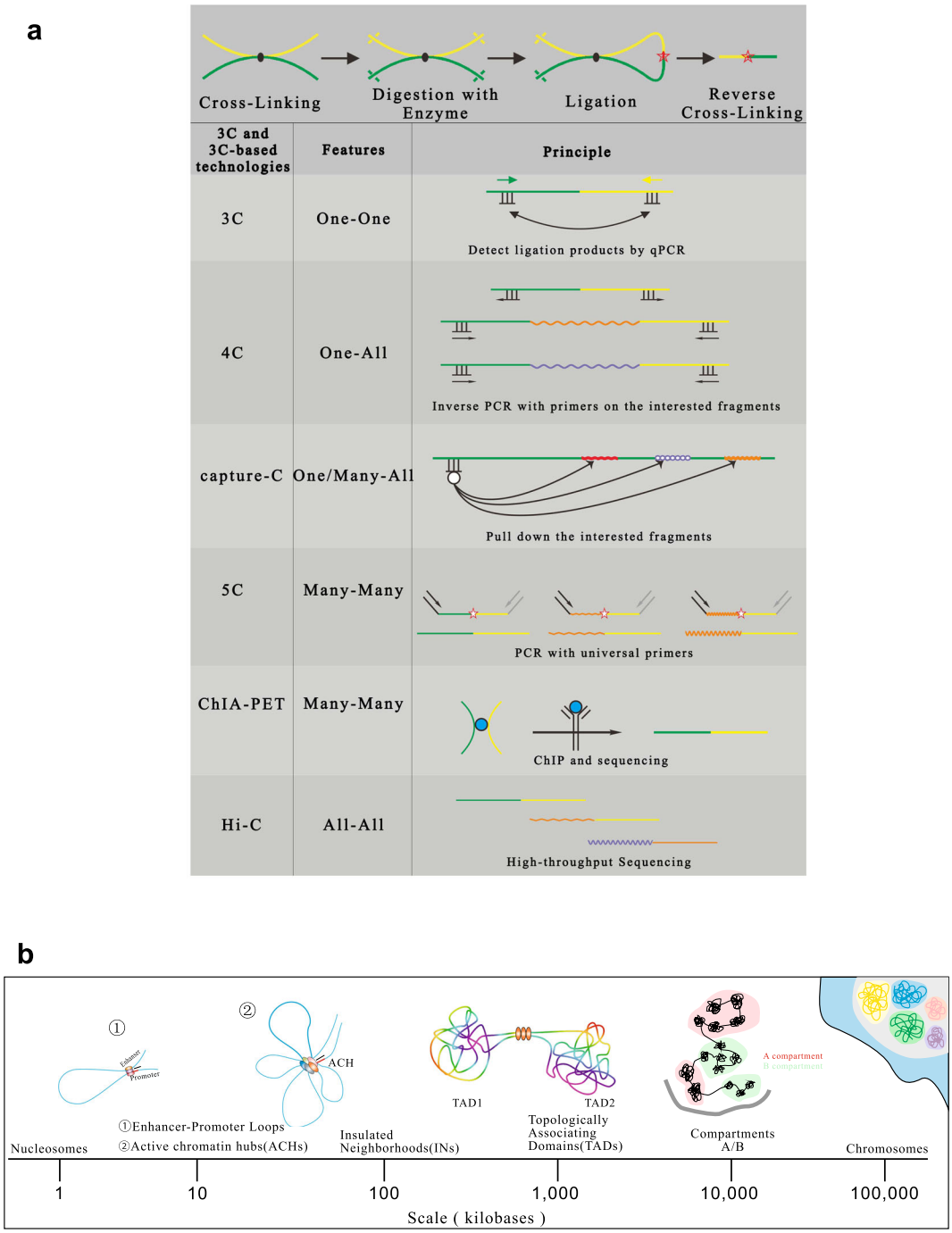

Fig. 1 Chromosome conformation capture technologies and three-dimensional chromosome structure. **a** Experimental principle and procedure of chromosome conformation capture (3C) and 3C-based technologies. Formaldehyde fixation for cross-linking is followed by digestion with a specific enzyme and ligation. Subsequently, the ligation products are enriched and contact frequencies are determined. **b** Hierarchical structures of 3D genome organization as inferred from 3C-based technologies. The hierarchical structures include chromosome territories (CTs), compartments A/B, topologically associated domains (TADs) (~ 100 kb–1000 kb). TADs consist of clusters of insulated neighborhoods (INs) (average size = ~200 kb) and chromatin loops (~ 10 kb–500 kb). Enhancer–promoter interaction loops and active chromatin hubs (ACHs) are constrained within INs

(Lieberman-Aiden et al. 2009). In 2014, researchers applied in situ Hi-C (Rao et al. 2014) to construct kilobase-resolution chromatin interaction maps for nine cell lines, enabling the detection of chromatin loops between enhancers and promoters. Recently single-cell Hi-C (Nagano et al. 2013, 2017; Ramani et al. 2017) methods reveal chromatin interactions within single cell and their heterogeneity across cells. Additionly, various Hi-C analysis and visualization tools have been developed to help us analyze and interpret Hi-C data (Table 2).

Although Hi-C technologies are powerful tools to unearth a huge amount of global chromatin interaction information, it suffers from low utilization of high-throughput sequencing data due to many invalid read pairs that do not reflect chromatin interactions. Targeted Chromosome Capture (T2C) (Kolovos et al. 2014) and Capture-C technology (Hughes et al. 2014) are developed to enrich the interaction fragments from chromosomal regions of interest by hybridizing ligation products to probes from these regions followed by biotin enrichment. In addition, Capture-Hi-C (Mifsud et al. 2015) can achieve fragment enrichment up to hundreds of fold, greatly improving the detection of local chromatin interactions of the genome regions of interest. However, the capture approaches do not offer a global view of chromatin interactions.

The technologies mentioned above, from 3C, 4C, 5C to Hi-C, T2C and Capture-Hi-C, are not aimed for capturing chromatin interactions mediated by specific proteins such as transcription factors and regulators. To identify such chromatin interactions, ChIA-PET (Fullwood et al. 2009) (chromatin interaction analysis by paired-end tag) is developed to combine 3C with chromatin immunoprecipitation (ChIP). To obtain a cost-efficient and more sensitive method than ChIA-PET, researchers further develop two strategies, PLAC-seq (proximity ligation-assisted ChIP-seq) (Fang et al. 2016) and HiChIP (protein-centric chromatin conformation assay) (Mumbach et al. 2016). PLAC-seq provides significantly improved efficiency and accuracy relative to ChIA-PET. Meanwhile, HiChIP can improve the yield of interaction-informative read pairs by over 10-fold and reduce the sample input requirement by over 100-fold relative to ChIA-PET.

Another modification of 3C-based methods is to capure the interactions between accessible chromatin regions. Currently, several sequencing methods including DNase-seq (Song and Crawford 2010), MNase-seq (Mieczkowski et al. 2016), ATAC-seq (Buenrostro et al. 2013) and ATAC-see (Chen et al. 2016) are based on nuclease-mediated assays and utilized for capturing gene regulatory elements which are often open chromatin. For example, DNase-seq makes use of DNase I sensitivity at open chromatin regions to enrich these sites from the whole genome and obtain a map of open chromatin regions. MNase-seq is developed to identify nucleosome occupancy and chromatin accessibility. Correspondingly, these methods are adapted to capture interactions between open chromatin regions. DNase Hi-C (Ma et al. 2015) characterizes long intergenic non-coding RNA (lincRNA) promoters and their interactions. With Micro-C (Hsieh et al. 2015), micrococcal

Table 2 Analysis and visualization tools for Hi-C data

Tool	Description of tools	References
TADbit	A pipeline for analysis and 3D modeling of 3C-based data.	Serra, et al. PLoS Comput Biol, 2017
Juicebox	A cloud-based visualization software for Hi-C data	Durand, et al. Cell Systems, 2016
HICUP	A pipeline for processing sequence data generated by Hi-C and Capture Hi-C (CHi-C) experiments	Wingett, et al. F1000Research, 2015
HiC-Pro	An optimized and flexible pipeline for processing Hi-C data from raw reads to normalized contact maps	Servant, et al. Genome Biol, 2015
Hi-Corrector	A fast, scalable and memory-efficient package for normalizing large-scale Hi-C data.	Li, et al. Bioinformatics, 2015
HiCNorm	Removing systematic biases in the raw Hi-C contact maps.	Hu M, et al. Bioinformatics, 2012
HiC-Box	A HiC data processing pipeline and visualizer, written mostly in Python.	https://github.com/koszullab/HiC-Box
Hiclib	A collection of tools to map, filter and analyze Hi-C data.	https://bitbucket.org/mirnylab/hiclib

nuclease (MNase) can fragment chromatin and reveal chromosome conformation at the nucleosome resolution.

In summary, since the chromosome conformation capture assay was invented in 2002, it has undergone many modifications, including 3C (one-to-one site), 4C (one-to-multiple sites), 5C (multiple-to-multiple genomic sites and high-throughput), whole genome (Hi-C, Micro-C) and targeted region analysis (ChIA-PET, Capture-C, Capture Hi-C). These technologies (shown in Table 1) have greatly advanced the study of spatial genome organization.

Novel findings on spatial genome organization

The 3C-based technologies have allowed researchers to obtain information on chromatin organization and interactions in many different cell types and organization scales. They reveal chromosome hierarchical structures (Gibcus and Dekker 2013; Bonev and Cavalli 2016) such as A/B compartments, topologically associated domains (TADs) with an average size of 1 Mb (Dixon et al. 2012; Crane et al. 2015; Nora et al. 2012), and chromatin loops with a median domain size of 185kb[6] (Fig. 1b). In general, euchromatin and heterochromatin regions are spatially separated within a chromosome territory (CT), and a chromosome is divided into compartments A and B. Compartment A usually consists of open and gene-rich chromatin regions, whereas compartment B consists of gene-poor and closed chromatin regions (Naumova and Dekker 2010). Consistently, compartments A and B correspond to higher and lower levels of gene expression, respectively. Moreover, analysis of high-resolution Hi-C data identified TADs and loops. In 2014, the whole genome chromatin loop maps of multiple human cell lines are reported (Rao et al. 2014). Chromatin loops can help form enhancer-promoter interactions and active chromatin hubs, which are structures composed of multiple enhancers and their interacting promoters (Add reference PMID:26686465). In addition, ChIA-PET data reveal the function of architectural proteins, such as CCCTC-binding factor (CTCF), cohesin and RNA polymerase II, which organize spatial chromatin structures for coordinated transcription (Tang et al. 2015). The importance of spatial chromatin structures is reflected by not only their impact on gene expression but also their conserved organization across species (Rao et al. 2014; Vietri Rudan et al. 2015). However, the relationship between the structure and function of the genome is not fully understood, and demands further 3D genome studies using cell lines, tissue samples and disease models.

3D genome disorganization in cancer

Cancer is a large group of malignant diseases caused by uncontrolled cell growth and spreading (Garraway and Lander 2013), characterized by various mutations in the genome, including point mutations, small insertions/deletions, chromosomal rearrangements, and copy number variations. In recent years, several systematic studies about the cancer genome have been performed (Garraway and Lander 2013). The Cancer Genome Atlas (TCGA) (Tomczak et al. 2015) has described landscapes of point mutations and structural variations in many types of cancer (Kandoth et al. 2013). Commonly mutated genes across different cancer types include TP53, KRAS, and PIK3CA. These genes are associated with major cell proliferation pathways, including MAPK signaling, PI(3)K signaling, and transcription regulation. Many studies also show that epigenetics are altered in cancer (Add reference PMID: 25421652). Although in recent decades we have learned much about how the genome sequence is mutated in many cancers, we know little about how the 3D genome is involved in cancer development.

Recently, several studies of the 3D cancer genome in breast cancer, prostate cancer, gliomas and multiple myeloma have been performed (Barutcu et al. 2015; Flavahan et al. 2016; Hnisz et al. 2016; Wu et al. 1937; Seaman et al. 2017; Taberlay et al. 2016). Researchers have found that the 3D genome is altered in cancers at different structural scales. Barutcu et al. performed Hi-C analysis on normal mammary epithelial (MCF-10A) and breast cancer (MCF-7) cell lines (Add reference PMID: 26415882). Their studies revealed that the 3D genome in cancer cells differs from that in normal cells. About 12% of genomic regions had A/B compartment switching in breast cancer when compared with normal cells. Furthermore, the switching from A compartment to B compartment was associated with downregulated gene expression. Compartment switching in the other direction is associated with upregulated gene

expression. In breast cancer cells, the inter-chromosomal interactions between the small and gene-rich chromosomes such as chromosome 16 through chromosome 22 had a lower interaction frequency than in normal cells. The intra-chromosomal interactions were also different: the telomeric and sub-telomeric regions interacted more frequently in normal cells than in breast cancer cells.

Subsequently, Taberlay et al. showed that the 3D genome is disorganized in prostate cancer (Taberlay et al. 2016). Specifically, they found that prostate cancer cells had more TAD boundaries and smaller average TAD length than normal cells. They also found that many cancer-specific domain boundaries occurred at regions with copy number variation. Moreover, the changes of long-range chromatin interactions were concordant with the changes of epigenetic modifications and gene expression. Wu et al. reported similar findings by integrating Hi-C, whole genome sequencing (WGS) and RNA-seq data to compare multiple myeloma cells and normal B cells (Add reference PMID:29203764).

Although studies in breast cancer and prostate cancer have provided evidence that the 3D genome is altered in cancers when compared with that in normal cells, these studies use normal and cancer cell lines and the association does not prove causation. Recent studies combining 3C and CRISPR/Cas9 techniques provided experimental evidence that disorganization in the 3D genome may cause cancer (Flavahan et al. 2016; Hnisz et al. 2016). Hnisz et al. applied cohesin ChIA-PET to T cell acute lymphoblastic leukemia (T-ALL) cells and identified insulated neighborhood regions (Hnisz et al. 2016). They found that recurrent microdeletions eliminated a neighborhood boundary and thus dysregulated T-ALL proto-oncogenes such as TAL1 and LMO2 (Fig. 2a). The authors used CRISPR/Cas9 to delete the CTCF-bound loop anchors surrounding these proto-oncogenes and caused their expression activation (Hnisz et al. 2016). In another study of gliomas, researchers found evidence that 3D genome reorganization is associated with insulator dysfuction and cancer (Flavahan et al. 2016). A major class of gliomas involves IDH mutations, which results in a CpG island methylator phenotype (G-CIMP) and causes hyper-methylation at CTCF binding sites. This leads to abnormal CTCF binding at a TAD boundary and results in aberrantly constitutive interaction between PDGFRA (a glioma oncogene) and its enhancer, thus activating the PDGFRA expression.

Copy number alterations affect the 3D cancer genome

Cancers are driven by mutations, including point mutations, indels, and somatic copy number alterations (SCNAs) such as duplications and deletions. SCNAs are common in many cancers (Beroukhim et al. 2010), and in some particular cancers, they are the driving mutation events (Ciriello et al. 2013). Previously, copy number mutations are considered to mainly affect coding genes: if duplication occurs at a gene then the gene may function as an oncogene; if deletion occurs, it functions as a tumor suppressor gene. However, through analyzing SCNAs across multiple cancer types, studies have found that less than one-quarter of recurrent SCNAs overlap with known cancer-driving genes (Zack et al. 2013). Some studies have found a new mechanism termed enhancer hijacking (Northcott et al. 2014) to explain the mechanism in which SCNAs cause cancer. A regulatory element such as an enhancer is rearranged by SCNAs and is juxtaposed with genes that it does not normally regulate. This leads to the misexpression of the juxtaposed genes, which may be related to the development of cancer. Therefore, SCNAs in cancer can affect the expression of genes in two ways: one is by a dosage effect and the other is by enhancer hijacking. By combining SCNAs and transcription profiling, we can readily identify dosage effect events in cancer (Add reference PMID: 21949216, 24088394), whereas it is more difficult to identify enhancer hijacking events occurring in cancer due to less knowledge about the 3D genome organization and enhancer locations in various cell types.

The identification of enhancer hijacking events requires the combination of genome alterations and 3D genome information. Weischenfeld et al. developed a novel method called cis expression structural alteration mapping (CESAM) (Weischenfeldt et al. 2017). CESAM integrates genome sequence, epigenetics, and 3D genome information and predicts the functional consequences of SCNA breakpoints of SCNAs by overlapping them with TADs. TADs are found to be largely conserved across cell types and there is some degree of correlated expression of genes within a TAD (Ali et al. 2016). TAD boundaries are bound by insulator proteins such as CTCF and prevent the interaction of genes and regulatory elements between different TADs. However, the disruption of TAD boundaries by SCNAs may change TAD structures and lead to the formation of a

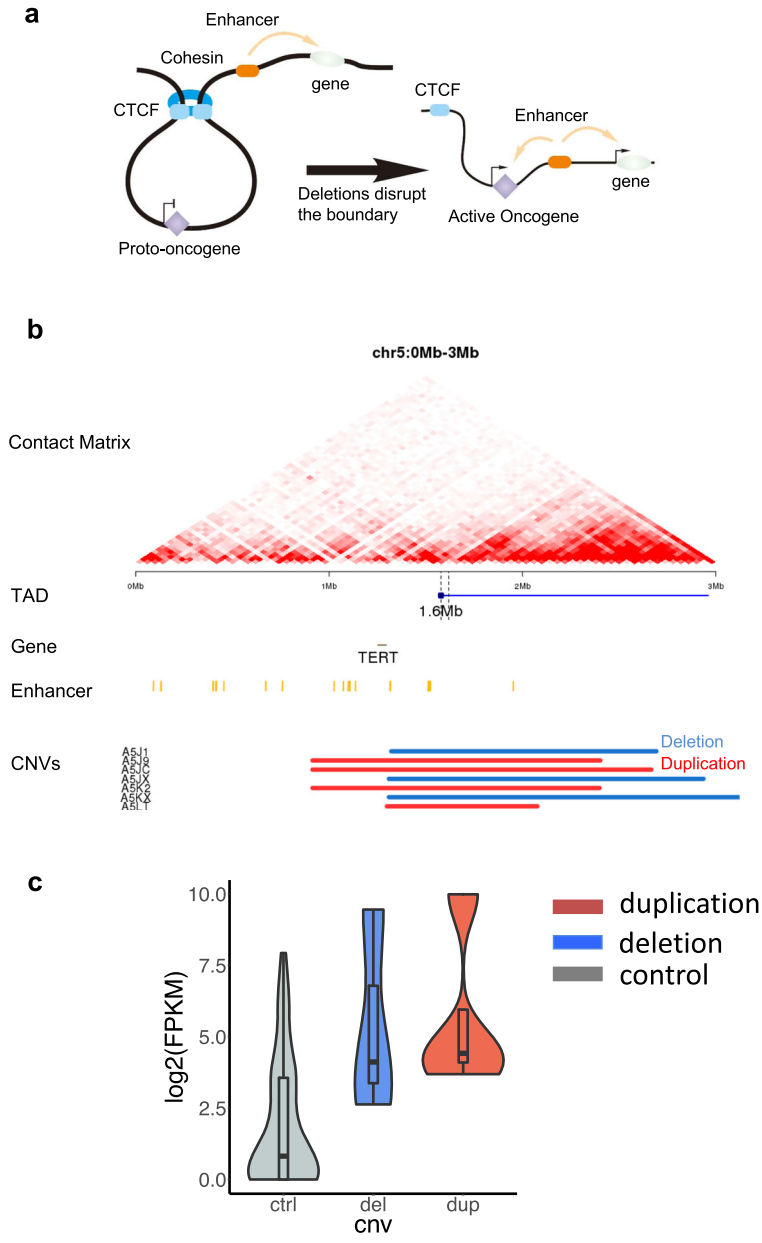

Fig. 2 Disruption of 3D chromosomal structures in cancer leads to abnormal gene expression. (**a**) Activation of proto-oncogenes by disrupting insulated loops. (**b**) Deletions or duplications disrupting TAD boundaries cause the abnormal expression of genes within or near the affected TADs. The figure displays the TERT locus and CNVs of adrenocortical carcinoma samples from TCGA. (**c**) TERT expression levels for samples with different types of CNVs and samples without CNVs. Both the samples with duplication (red) and the samples with deletion (blue) show higher gene expression compared to the samples without CNVs (grey, used as control)

new TADs (Franke et al. 2016). Consequently, the interaction between genes and regulatory elements located within and nearby the altered TADs may change. Making assumption of this mechanism, CESAM evaluates the impact of SCNAs on local genome structures by mapping SCNA breakpoints relative to TADs (Fig. 2b). To identify potential enhancer hijacking events, CESAM builds up a linear regression model correlating SCNA events, TAD boundaries and gene expression to determine whether the expression of a gene is affected by nearby SCNAs through altered TAD structures (Fig. 2c). The authors analyzed 7416 cancer samples from the TCGA data portal, and predicted 18 candidate genes that are upregulated in cancer samples due to SCNA-induced TAD reorganization, including IRS4 and IGF2. This work provides a good illustration of how to apply the 3D genome information to identify novel mechanisms of cancer-driving mutations.

By integrating different techniques such as Hi-C, ChIA-PET, RNA-seq, and CRISPR/Cas9, the functional

consequences of many noncoding mutations and structural variations in cancer may be inferred from the 3D genome perspective. We are optimistic that studies with additional cancer types and clinical cancer samples will identify more cancer alterations that exert their influences through 3D genome disorganization.

3D genome disorganization in other diseases

The 3D genome structure is important not only in cancer but also in other diseases, such as congenital limb malformations (Lupianez et al. 2015), autoimmune diseases (Martin et al. 2015), and cooks syndrome and sex reversal (Franke et al. 2016). In these diseased conditions, there are germline mutations in the genome, such as chromosomal rearrangements (CRs; some CRs cause copy number variations) and single-nucleotide variations (SNVs). CR events (including deletion, duplication, insertion, inversion, translocation) are common structural variations in the human genome but their functional impact is often elusive (Shrivastav et al. 2008). They usually lead to diseases by disrupting gene or protein structures, but may also affect gene expression by altering chromosomal 3D structures (Taberlay et al. 2016; Lupianez et al. 2015). A recent study showed that CR events cause polydactyly by altering 3D chromosomal organization (Lupianez et al. 2015). A model for the pathogenicity of CR events is shown in Fig. 3. By using CRISPR/Cas9 and mouse models, the authors further confirmed the causal roles of 3D genome disorganization in developmental diseases.

Genome-wide association studies (GWASs) have been successfully used to identify genetic variants associated with complex diseases (Li et al. 2017). However, how these non-coding variants influence diseases is often unclear. Most disease-associated SNPs are located in non-coding chromosomal regions and overlap with

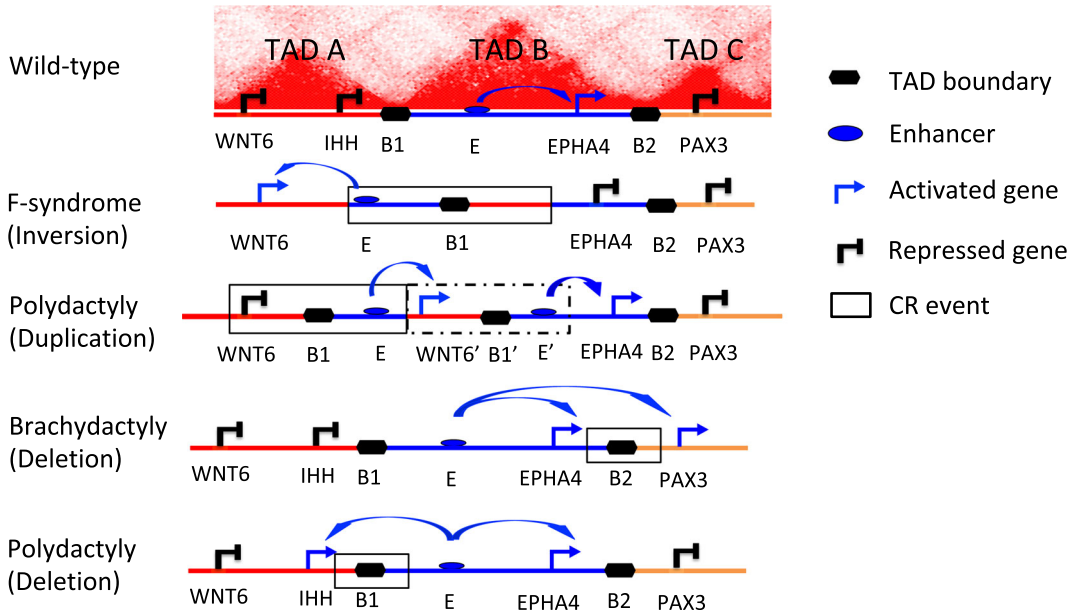

Fig. 3 Model for the pathogenicity of CR events that disrupt TADs. A model for CR events at the WNT6, IHH, EPHA4, and PAX3 locus in brachydactyly, polydactyly, and F-syndrome diseases, which disrupt the boundaries of a TAD containing limb-developing enhancers. The wild-type chromosome conformation shows the structure of three TADs, separated by boundary elements. The activity of an enhancer (E) is restricted to gene EPHA4 located inside TAD B. In the F-syndrome with the inversion of a chromosome region, E is moved out of TAD B and placed near gene WNT6 located in TAD A, and the boundary is now on the right side of E. This results in ectopic interaction between E and WNT6 but prevents the normal interaction between E and EPHA4. In the polydactyly disease with a duplication event (dashed rectangle), E is placed next to the duplicated WNT6 within a newly created TADf, resulting in their interaction and abnormal expression of WNT6. In the brachydactyly disease with a deletion event, the deletion removes the boundary B2 and portions of TAD B and TAD C, and E is able to interact with both EPHA4 and PAX3, resulting in abnormal expression of PAX3. In a similar way, the expression of IHH is abnormal in polydactyly disease with a deletion event involving the boundary B1. Figure adapted from Li et al. (Add reference PMID: 27734896) and Lupianez et al. (Add reference PMID: 25959774)

enhancers (Fairfax et al. 2014; Farh et al. 2015). This motivates the efforts to identify which genes form physical interactions with the chromatin regions containing disease-associated GWAS variants. Capture Hi-C is useful in this context as it can help us to identify these promoter-enhancer interactions cost-effectively. For example, a recent study used this approach to identify the interactions between the SNPs associated with four autoimmune diseases and their potential functional target genes in B cells and T cells (Martin et al. 2015). Looping interactions identified by Capture Hi-C provided evidence that many disease-associated SNPs do not interact with the nearest genes but with genes often several megabases away. Specifically, the region of SNPs associated with rheumatoid arthritis (RA) has strong physical interaction with the promoter of gene AZI2 located 640 kb away, which is involved in the NF-κB pathway activity.

To integrate and visualize the relationship between disease-associated CRs and the 3D chromosomal structure, Li et al. developed a web server to query a disease-associated CR event or a gene of intersest (3Disease Browser, http://3dgb.cbi.pku.edu.cn/disease/) (Li et al. 2016). The 3Disease Browser integrates Hi-C data, annotation tracks, and 3D chromatin models overlaid with epigenetic information. Other 3D genome browsers have also been developed to integrate and visualize multiple data types (see Table 3 for a comparison of 3D genome browsers).

Clinical and multi-omics applications of 3D genome technologies

There are now many omics technologies available that can help us obtain a deeper insight into diseases, such as RNA-seq and WGS. 3D genome technologies such as Hi-C add to these omics technologies by detecting 3D genome structural changes in diseases and inferring their impact on gene expression. We take clinical cancer research as an example to illustrate the application of 3D genome technologies (Fig. 4). Paired normal and cancer tissue samples are obtained from cancer patients for omics experiments such as Hi-C and RNA-seq. Many diseases including cancer are associated with chromosome structural variations, such as copy number variations (CNVs) and translocations. In clinical research, we usually perform WGS to detect such structural variations, but we and others have found that Hi-C data can also be used to detect and confirm structural variations (Wu et al. 1937; Chakraborty and Ay 2017). In a recent study (Chakraborty and Ay 2017), researchers showed that Hi-C data can detect large-scale CNVs (> 1 Mb) and translocations accurately when compared with those detected by WGS. Therefore, Hi-C data can not only provide us with 3D structural information but also CNVs and translocations. Through an integrative multi-omics analysis (Figure 4), we expect to determine significant differences between normal and cancer samples, correlate the alterations between different omics types, and infer the causes and consquences of these genomic alterations.

With technological progress, 3D genome approaches are promising to be applied to clinical and therapeutic research. Almassalha et al. used drugs to alter the chromatin assembly density, which prevented the evolution of cancer cells and enhanced the effectiveness of anticancer therapy (Almassalha et al. 2017). This study suggests that therapeutic treatment may be achieved by modifying the 3D structures of chromatin and genome. It is exciting to explore editing the 3D genome for therapeutic purposes when the relationship between chromatin structures and diseases are better understood (Sachdeva et al. 2015; Huang and Wu 2016).

Single cell Hi-C and future development of 3D genome technologies

There are several challenges facing the application of 3D genome technologies in clinical research. For example, to carry out in situ Hi-C, millions of cells are required; however, for clinical samples, the sample volume of an interested cell type is much smaller. It is less studied whether a smaller volume of cells can be used to detect chromatin interactions from clinical samples. In addition, other challenges when handling clinical samples are similar to traditional genomic studies, such as sufficiently clean separation of tumor and normal cells and the removal of non-interested cell types.

In addition, the 3D genome technologies represented by Hi-C suffer from low resolution and biases, which may be caused by uneven fragmentation, PCR

Table 3 Comparison of 3D genome browsers

Web server or software	Hi-C heatmap display	Annotation tracks	Gene search	Disease search	GWAS variants search	3D model display	Website
3Disease Browser (Li et al. 2016)	✓	✓	✓	✓		✓	http://3dgb.cbi.pku.edu.cn/disease
Hi-Browse v1.6 (Paulsen et al. 2014)	✓	✓	✓				https://hyperbrowser.uio.no/3d/
ChromContact (Sato and Suyama 2015)		✓	✓		✓		http://bioinfo.sls.kyushu-u.ac.jp/chromcontact/
HiView (Xu et al. 2016)		✓	✓	✓	✓		http://www.unc.edu/~yunmli/HiView/
Hi-C data Browser (Lieberman-Aiden et al. 2009) (Dekker lab)	✓						http://hic.umassmed.edu/welcome/welcome.php
HiC-3DViewer	✓	✓				✓	http://bioinfo.au.tsinghua.edu.cn/member/nadhir/HiC3DViewer
Hi-C data Browser (Yue lab)	✓	✓					http://yuelab.org/hi-c/database.php
WashU EpiGenome Browser (Zhou et al. 2013) (Wang lab)	✓	✓	✓		✓		http://vizhub.wustl.edu/
4DGenome Browser (Teng et al. 2015)		✓	✓				http://4dgenome.int-med.uiowa.edu
Genomic HyperBrowser (Sandve et al. 2010)		✓					https://hyperbrowser.uio.no/hb/
GMOL (Nowotny et al. 2016)						✓	https://sourceforge.net/projects/gmol/
Genome3D (Lewis et al. 2015)						✓	http://www.genome3d.eu
Tadkit		✓				✓	http://sgt.cnag.cat/3dg/tadkit/

amplification bias, noisy ligation or other factors (Yaffe and Tanay 2011). To address these issues, the Hi-C technology has been extended and various improved versions have been developed. In bridge linker Hi-C (BL-Hi-C), 20 bp biotin-labeled bridge linkers are added to the fragmented DNA solution for two-step ligations and reduced ligation noise(Liang et al. 2017). Linear PCR methods (Chen et al. 2017) may be used reduce PCR amplification bias (Add reference PMID: 28408603). Improving the resolution and reducing the noise of Hi-C technologies will enable researchers to investigate subtle changes of 3D genome structures in biological and disease processes.

Single-cell Hi-C (scHi-C) (Nagano et al. 2013, 2017; Ramani et al. 2017) is another important direction for the development of Hi-C technologies. On the one hand, the number of cells that can be obtained from embryonic development or some rare diseases is very limited. On the other hand, the 3D genome structure of cancer cells may be very heterogeneous. The development of scHi-C can not only verify the results of bulk Hi-C but also aid the study of diseases such as cancer by revealing the molecular heterogeneity behind them. The technical challenge of scHi-C is how to reduce DNA loss and noise during experimental steps. It is difficult to achieve the balance between the two goals because one can lose true interaction reads when filtering out noisy interactions. One effective way to reduce DNA loss is to simplify the experimental workflow, since every step could cause DNA loss. The first scHi-C technology was established in 2013 (Nagano et al. 2013). In this approach, Nagano et al. first cut DNA by restriction enzyme (Bgl II or Dpn II) and then labelled the fragments with biotin in each nucleus. Each cell nucleus was manually picked out after proximity ligation, and then the fragment was captured by biotin pull-down, cut by Alu I, amplified by adapter ligation PCR, and finally

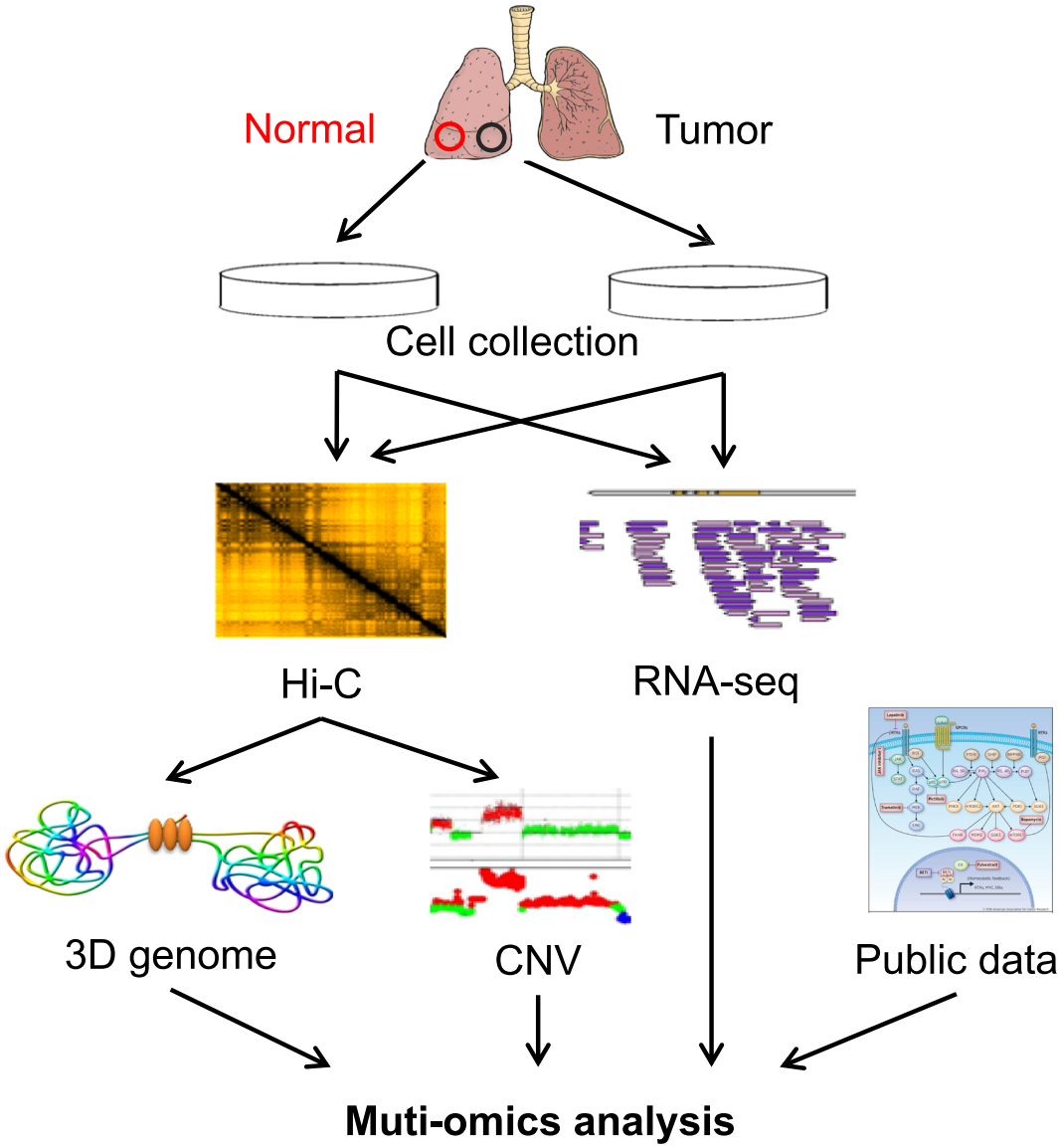

Fig. 4 Schematic representation of the pipeline for applying 3C technologies to clinic research. Paired normal and cancer tissue samples are obtained from cancer patients for multi-omics experiments such as Hi-C and RNA-seq followed by integrative analysis

sequenced (Nagano et al. 2013). In 2017, Nagano et al. introduced flow cytometry sorting instead of manual nucleus isolation and used Tn5-transposase tagmentation to substitute for the last several steps including Alu I digestion, end repairing, and adapter ligation (Nagano et al. 2017). These advances further simplified the experimental workflow of scHi-C and greatly improved the number of sequenced DNA reads (Table 4). Flyamer et al. developed another scHi-C technology (Flyamer et al. 2017). They did not biotin-labeled DNA fragments but instead amplified the whole genome after DNA ligation and reverse cross-linking (Flyamer et al. 2017). The valid read pairs were then filtered using computational approaches. Table 4 summarizes various scHi-C protocols. Overall, the number of valid read pairs obtained from current scHi-C technologies falls short of the level required to draw a reliable chromatin contact map of single cells. We expect future development of more sophisticated scHi-C technologies will provide sufficient number of interaction reads and minimal noise.

Table 4 Summary of single cell Hi-C technologies

Protocol	Technique summary	Median number of captured contacts per cell
Single cell Hi-C technologies		
Nagano. et al. 2015	Cross-linking → restriction digestion → biotin fill-in → ligation → isolate single nucleus manually → reversed cross-linking → biotin pull down → fragmentation by Alu I → adapter ligation → PCR → sequencing	18,166
Nagano. et al. 2017	Cross-linking → restriction digestion → biotin fill-in → ligation → isolate single nucleus by FACS → reversed cross-linking → biotin pull down → Tn5 transposase fragmentation → PCR → sequencing	127,233
Flyamer. et al. 2017	Cross-linking → restriction digestion → ligation → single nucleus isolation → reversed cross-linking → genome PCR → purification and shearing → adapter ligation → sequencing Cross-linking → single nucleus isolation → restriction digestion → ligation → reversed cross-linking → genome PCR → purification and shearing → adapter ligation → sequencing	131,384~213,533 (Different cell types)
Ramani. et al. 2017	Cross-linking → restriction digestion → biotinylated barcode ligation → ligation → isolate 10 nucleus per well → sequencing adapter ligation → libraries combining → biotin pull down → sequencing	~10,000
Stevens. et al. 2017)	Cross-linking → single nucleus isolation → restriction digestion → biotin fill-in → ligation → reversed cross-linking → biotin pull down → adapter ligation → libraries combining → sequencing	74,885

Hi-C can reveal genome-wide chromatin interactions, but the resolution of interaction map depends on the depth of sequencing, and the cost of sequencing could be huge for whole-genome, high-resolution interaction maps. For some diseases such as polydactyly diseases (Zack et al. 2013) discussed above, interaction maps of local regions containing suspected disease genes provide enough information. Under such circumstance, targeted Chromosome Capture (T2C) (Kolovos et al. 2014) and Capture-C technology (Hughes et al. 2014) are good alternatives to Hi-C. These two technologies capture specific regions' chromatin interactions and enable researchers to obtain interaction maps with sufficient resolution at affordable cost.

Constrained by the characteristics of next generation sequencing, the highly repetitive genome regions of chromatin cannot be aligned to the reference genome accurately, but these highly repetitive regions may have important functions. For instance, telomeres play an important role in cancer and aging (Gilley et al. 2005). Currently we have little understanding of the spatial genome organization of these regions. With the growing applications of the third generation sequencing, repetitive regions can be sequenced accurately (Chin et al. 2013). Technologies combining the third generation sequencing with chromatin capture technologies are likely to be developed to better reveal the chromatin conformation structure of highly repetitive regions.

In conclusion, the 3D genome technologies have greatly facilitated the study of developmental diseases and complex diseases. Complex diseases such as cancer arise not from the mutations of a single gene but from the changes of complex interactions between genes and their regulatory elements (Bailey et al. 2014; Forbes et al. 2015). Studying the chromatin conformation changes in diseases can help to understand these interactions and hint at novel predictive biomarkers or treatment targets. In the future, single-cell, high-throughput and multi-omics 3D genome assays will be developed, allowing us to obtain more in-depth information to understand fundamental and dynamic structures of genome organization in normal and diseased conditions.

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Author contribution Ruifeng Li designed and supervised this review. Yingping Hou was responsible for the section on 3D genome technology and novel findings. Yuting Liu and Pengze Wu was responsible for the section on 3D genome reorganization in cancer and clinical applications. Ruifeng Li was responsible for the section on 3D genome reorganization in other diseases. Jingbo Gan was responsible for the section on single cell Hi-C and future directions. C.L. participated in writing.

References

Ali T, Renkawitz R, Bartkuhn M. Insulators and domains of gene expression. Curr Opin Genet Dev. 2016;37:17–26. https://doi.org/10.1016/j.gde.2015.11.009.

Almassalha LM, et al. Macrogenomic engineering via modulation of the scaling of chromatin packing density. Nat Biomed Eng. 2017;1:902–13. https://doi.org/10.1038/s41551-017-0153-2.

Bailey JN, Pericak-Vance MA, Haines JL. The impact of the human genome project on complex disease. Genes. 2014;5:518–35. https://doi.org/10.3390/genes5030518.

Barutcu AR, et al. Chromatin interaction analysis reveals changes in small chromosome and telomere clustering between epithelial and breast cancer cells. Genome Biol. 2015;16:214. https://doi.org/10.1186/s13059-015-0768-0.

Beroukhim R, et al. The landscape of somatic copy-number alteration across human cancers. Nature. 2010;463:899–905. https://doi.org/10.1038/nature08822.

Bonev B, Cavalli G. Organization and function of the 3D genome. Nat Rev Genet. 2016;17:661–78. https://doi.org/10.1038/nrg.2016.112.

Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. Nat Methods. 2013;10:1213–8. https://doi.org/10.1038/nmeth.2688.

Chakraborty A, Ay F. Identification of copy number variations and translocations in cancer cells from Hi-C data. Bioinformatics. 2017; https://doi.org/10.1093/bioinformatics/btx664.

Chaudhary K, Chattopadhyay A, Pratap D. The evolution of CRISPR/Cas9 and their cousins: hope or hype? Biotechnol Lett. 2018;40:465–77. https://doi.org/10.1007/s10529-018-2506-7.

Chen X, et al. ATAC-see reveals the accessible genome by transposase-mediated imaging and sequencing. Nat Methods. 2016;13:1013–20. https://doi.org/10.1038/nmeth.4031.

Chen C, et al. Single-cell whole-genome analyses by linear amplification via transposon insertion (LIANTI). Science. 2017;356:189–94. https://doi.org/10.1126/science.aak9787.

Chin CS, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods. 2013;10:563–9. https://doi.org/10.1038/nmeth.2474.

Ciriello G, et al. Emerging landscape of oncogenic signatures across human cancers. Nat Genet. 2013;45:1127–33. https://doi.org/10.1038/ng.2762.

Crane E, et al. Condensin-driven remodelling of X chromosome topology during dosage compensation. Nature. 2015;523:240–4. https://doi.org/10.1038/nature14450.

Dekker J, Rippe K, Dekker M, Kleckner N. Capturing Chromosome Conformation. Science. 2002; https://doi.org/10.1126/science.1067799.

Dixon JR, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature. 2012;485:376–80. https://doi.org/10.1038/nature11082.

Dostie J, et al. Chromosome Conformation Capture Carbon Copy (5C): a massively parallel solution for mapping interactions between genomic elements. Genome Res. 2006;16:1299–309. https://doi.org/10.1101/gr.5571506.

Durand NC, et al. Juicebox provides a visualization system for Hi-C contact maps with unlimited zoom. Cell Syst. 2016;3:99–101. https://doi.org/10.1016/j.cels.2015.07.012.

Fairfax BP, et al. Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. Science. 2014;343:1246949. https://doi.org/10.1126/science.1246949.

Fang R, et al. Mapping of long-range chromatin interactions by proximity ligation-assisted ChIP-seq. Cell Res. 2016;26:1345–8. https://doi.org/10.1038/cr.2016.137.

Farh KK, et al. Genetic and epigenetic fine mapping of causal autoimmune disease variants. Nature. 2015;518:337–43. https://doi.org/10.1038/nature13835.

Flavahan WA, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature. 2016;529:110–4. https://doi.org/10.1038/nature16490.

Flyamer IM, et al. Single-nucleus Hi-C reveals unique chromatin reorganization at oocyte-to-zygote transition. Nature. 2017;544:110–4. https://doi.org/10.1038/nature21711.

Forbes SA, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res. 2015;43:D805–11. https://doi.org/10.1093/nar/gku1075.

Franke M, et al. Formation of new chromatin domains determines pathogenicity of genomic duplications. Nature. 2016;538:265–9. https://doi.org/10.1038/nature19800.

Fullwood MJ, et al. An oestrogen-receptor-alpha-bound human chromatin interactome. Nature. 2009;462:58–64. https://doi.org/10.1038/nature08497.

Garraway LA, Lander ES. Lessons from the cancer genome. Cell. 2013;153:17–37. https://doi.org/10.1016/j.cell.2013.03.002.

Gibcus JH, Dekker J. The hierarchy of the 3D genome. Mol Cell. 2013;49:773–82. https://doi.org/10.1016/j.molcel.2013.02.011.

Gilley D, Tanaka H, Herbert BS. Telomere dysfunction in aging and cancer. Int J Biochem Cell Biol. 2005;37:1000–13. https://doi.org/10.1016/j.biocel.2004.09.003.

Hnisz D, et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. Science. 2016;351:1454–8. https://doi.org/10.1126/science.aad9024.

Hsieh TH, et al. Mapping nucleosome resolution chromosome folding in yeast by micro-C. Cell. 2015;162:108–19. https://doi.org/10.1016/j.cell.2015.05.048.

Hu M, et al. HiCNorm: removing biases in Hi-C data via Poisson regression. Bioinformatics. 2012;28:3131–3. https://doi.org/10.1093/bioinformatics/bts570.

Huang H, Wu Q. CRISPR double cutting through the labyrinthine architecture of 3D genomes. J Genet Genomics. 2016;43:273–88. https://doi.org/10.1016/j.jgg.2016.03.006.

Hughes JR, et al. Analysis of hundreds of cis-regulatory landscapes at high resolution in a single, high-throughput experiment. Nat Genet. 2014;46:205–12. https://doi.org/10.1038/ng.2871.

Kandoth C, et al. Mutational landscape and significance across 12 major cancer types. Nature. 2013;502:333–9. https://doi.org/10.1038/nature12634.

Kolovos P, van de Werken HJG, Kepper N, Zuin J, Brouwer RWW, Kockx CEM, et al. Targeted chromatin capture (T2C): a novel high resolution high throughput method to detect genomic interactions and regulatory elements. Epigenetics Chromatin. 2014;7:10. https://doi.org/10.1186/1756-8935-7-10.

Lewis TE, et al. Genome3D: exploiting structure to help users understand their sequences. Nucleic Acids Res. 2015;43:D382–6. https://doi.org/10.1093/nar/gku973.

Li W, Gong K, Li Q, Alber F, Zhou XJ. Hi-corrector: a fast, scalable and memory-efficient package for normalizing large-scale hi-C data. Bioinformatics. 2015;31:960–2. https://doi.org/10.1093/bioinformatics/btu747.

Li R, Liu Y, Li T, Li C. 3Disease browser: a web server for integrating 3D genome and disease-associated chromosome rearrangement data. Sci Rep. 2016;6:34651. https://doi.org/10.1038/srep34651.

Li Z, Chen J, Yu H, He L, Xu Y, Zhang D, et al. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. Nat Genet. 2017;49(11):1576-1583. https://doi.org/10.1038/ng.3973.

Liang Z, et al. BL-Hi-C is an efficient and sensitive approach for capturing structural and regulatory chromatin interactions. Nat Commun. 2017;8:1622. https://doi.org/10.1038/s41467-017-01754-3.

Lieberman-Aiden E, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science. 2009;326:289–93. https://doi.org/10.1126/science.1181369.

Lupianez DG, et al. Disruptions of topological chromatin domains cause pathogenic rewiring of gene-enhancer interactions. Cell. 2015;161:1012–25. https://doi.org/10.1016/j.cell.2015.04.004.

Ma W, et al. Fine-scale chromatin interaction maps reveal the cis-regulatory landscape of human lincRNA genes. Nat Methods. 2015;12:71–8. https://doi.org/10.1038/nmeth.3205.

Martin P, et al. Capture Hi-C reveals novel candidate genes and complex long-range interactions with related autoimmune risk loci. Nat Commun. 2015;6:10069. https://doi.org/10.1038/ncomms10069.

Mieczkowski J, et al. MNase titration reveals differences between nucleosome occupancy and chromatin accessibility. Nat Commun. 2016;7:11485. https://doi.org/10.1038/ncomms11485.

Mifsud B, Tavares-Cadete F, Young AN. Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. Nat Genet. 2015; https://doi.org/10.1038/ng.3286.

Mumbach MR, et al. HiChIP: efficient and sensitive analysis of protein-directed genome architecture. Nat Methods. 2016; https://doi.org/10.1038/nmeth.3999.

Nagano T, et al. Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. Nature. 2013;502:59–64. https://doi.org/10.1038/nature12593.

Nagano T, et al. Single-cell Hi-C for genome-wide detection of chromatin interactions that occur simultaneously in a single cell. Nat Protoc. 2015;10:1986–2003. https://doi.org/10.1038/nprot.2015.127.

Nagano T, et al. Cell-cycle dynamics of chromosomal organization at single-cell resolution. Nature. 2017;547:61–7. https://doi.org/10.1038/nature23001.

Naumova N, Dekker J. Integrating one-dimensional and three-dimensional maps of genomes. J Cell Sci. 2010;123:1979–88. https://doi.org/10.1242/jcs.051631.

Nora EP, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature. 2012;485:381–5. https://doi.org/10.1038/nature11049.

Northcott PA, et al. Enhancer hijacking activates GFI1 family oncogenes in medulloblastoma. Nature. 2014;511:428–34. https://doi.org/10.1038/nature13379.

Nowotny J, et al. GMOL: an interactive tool for 3D genome structure visualization. Sci Rep. 2016;6:20802. https://doi.org/10.1038/srep20802.

Paulsen J, et al. HiBrowse: multi-purpose statistical analysis of genome-wide chromatin 3D organization. Bioinformatics. 2014;30:1620–2. https://doi.org/10.1093/bioinformatics/btu082.

Ramani V, et al. Massively multiplex single-cell Hi-C. Nat Methods. 2017; https://doi.org/10.1038/nmeth.4155.

Rao SS, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. Cell. 2014;159:1665–80. https://doi.org/10.1016/j.cell.2014.11.021.

Sachdeva M, et al. CRISPR/Cas9: molecular tool for gene therapy to target genome and epigenome in the treatment of lung cancer. Cancer Gene Ther. 2015;22:509–17. https://doi.org/10.1038/cgt.2015.54.

Sandve GK, et al. The Genomic HyperBrowser: inferential genomics at the sequence level. Genome Biol. 2010;11:R121. https://doi.org/10.1186/gb-2010-11-12-r121.

Sato T, Suyama M. ChromContact: a web tool for analyzing spatial contact of chromosomes from Hi-C data. BMC Genomics. 2015;16:1060. https://doi.org/10.1186/s12864-015-2282-x.

Seaman L, et al. Nucleome analysis reveals structure-function relationships for colon cancer. Mol Cancer Res. 2017;15:821–30. https://doi.org/10.1158/1541-7786.MCR-16-0374.

Serra F, et al. Automatic analysis and 3D-modelling of Hi-C data using TADbit reveals structural features of the fly chromatin colors. PLoS Comput Biol. 2017;13:e1005665. https://doi.org/10.1371/journal.pcbi.1005665.

Servant N, et al. HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. Genome Biol. 2015;16:259. https://doi.org/10.1186/s13059-015-0831-x.

Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. Cell Res. 2008;18:134–47. https://doi.org/10.1038/cr.2007.111.

Simonis M, et al. Nuclear organization of active and inactive chromatin domains uncovered by chromosome conformation

capture-on-chip (4C). Nat Genet. 2006;38:1348–54. https://doi.org/10.1038/ng1896.

Song, L. & Crawford, G. E. DNase-seq: a high-resolution technique for mapping active gene regulatory elements across the genome from mammalian cells. Cold Spring Harb Protoc. 2010. https://doi.org/10.1101/pdb.prot5384.

Stevens TJ, et al. 3D structures of individual mammalian genomes studied by single-cell Hi-C. Nature. 2017;544:59–64. https://doi.org/10.1038/nature21429.

Taberlay PC, et al. Three-dimensional disorganisation of the cancer genome occurs coincident with long range genetic and epigenetic alterations. Genome Res. 2016; https://doi.org/10.1101/gr.201517.115.

Tang Z, et al. CTCF-mediated human 3D genome architecture reveals chromatin topology for transcription. Cell. 2015;163:1611–27. https://doi.org/10.1016/j.cell.2015.11.024.

Teng L, He B, Wang J, Tan K. 4DGenome: a comprehensive database of chromatin interactions. Bioinformatics. 2015;31:2560–4. https://doi.org/10.1093/bioinformatics/btv158.

Tomczak K, Czerwinska P, Wiznerowicz M. The cancer genome atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn). 2015;19:A68–77. https://doi.org/10.5114/wo.2014.47136.

Van de Werken HJ, et al. Robust 4C-seq data analysis to screen for regulatory DNA interactions. Nat Methods. 2012;9:969–72. https://doi.org/10.1038/nmeth.2173.

Vietri Rudan M, et al. Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. Cell Rep. 2015;10:1297–309. https://doi.org/10.1016/j.celrep.2015.02.004.

Weischenfeldt J, et al. Pan-cancer analysis of somatic copy-number alterations implicates IRS4 and IGF2 in enhancer hijacking. Nat Genet. 2017;49:65–74. https://doi.org/10.1038/ng.3722.

Wingett S, et al. HiCUP: pipeline for mapping and processing Hi-C data. F1000Research. 2015;4:1310. https://doi.org/10.12688/f1000research.7334.1.

Wu P, et al. 3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. Nat Commun. 2017;8:1937. https://doi.org/10.1038/s41467-017-01793-w.

Xu Z, et al. HiView: an integrative genome browser to leverage Hi-C results for the interpretation of GWAS variants. BMC Res Notes. 2016;9:159. https://doi.org/10.1186/s13104-016-1947-0.

Yaffe E, Tanay A. Probabilistic modeling of Hi-C contact maps eliminates systematic biases to characterize global chromosomal architecture. Nat Genet. 2011;43:1059–65. https://doi.org/10.1038/ng.947.

Zack TI, et al. Pan-cancer patterns of somatic copy number alteration. Nat Genet. 2013;45:1134–40. https://doi.org/10.1038/ng.2760.

Zhou X, et al. Exploring long-range genome interactions using the WashU Epigenome Browser. Nat Methods. 2013;10:375–6. https://doi.org/10.1038/nmeth.2440.