

Three-Dimensional Genomics: A New Perspective and Therapeutic Strategies in Prostate Cancer Research

Yixiang Sun *

School of Huazhong Agriculture University, Wuhan, China

* Corresponding author: zamperini@webmail.hzau.edu.cn

Abstract. Prostate cancer (PC) poses significant health risks to men globally. Enhancing our understanding of prostate cancer biology is crucial for facilitating early diagnosis and effective treatment strategies. A multitude of high-throughput sequencing studies, encompassing whole genome resequencing, transcriptome sequencing, and genome-wide association studies, have unearthed vital point mutations, structural variations, and epigenomic alterations linked to prostate cancer. These findings have significantly enriched our knowledge of the genomic framework for prostate cancer. Still, these investigations have predominantly centered on the one- or two-dimensional landscape of the genome. Research in three-dimensional genomics underscores the critical role of the genome's three-dimensional spatial structure in maintaining normal cellular functions. Additionally, it demonstrates that dysregulation of key genes in numerous cancers relates to the chromatin's spatial organization across various levels. This article explores the intricate three-dimensional architecture of chromosomes. It outlines the progressive development of techniques used in three-dimensional genomic research and synthesizes the application of these techniques in the study of prostate cancer biology. Furthermore, it proposes potential therapeutic strategies for prostate cancer.

Keywords: Three-dimensional genomics; Prostate cancer; Chromatin structure.

1. Introduction

Three-dimensional genomics, a burgeoning field, seeks to elucidate the three-dimensional spatial structure and function of genomes. This realm places great emphasis on comprehending the spatial arrangement of genomic sequences within the cellular nucleus' tumultuous milieu, further analyzing the significant biological impact of such formations on fundamental cellular processes, such as the conservation of DNA structural integrity throughout replication and recombination, along with the regulated control of gene expression. Using advanced tools such as the chromatin conformation capture (3C) technology, researchers have been able to significantly expand the scope of 3D genomics research. The resources provided by the utilization of 3C, along with its advanced derivatives such as Hi-C and ChIA-PET, open up vast opportunities for an in-depth examination of the unique three-dimensional genomic patterns exclusive to various cancer types. This allows for an understanding of the spatial organization of cancer genomes, chromatin interaction patterns, transcriptional regulation, and the underlying mechanisms behind distinct biological traits.

Prostate cancer, known for its high hereditary risk, has become a pervasive health issue in society. As uncovered through Genome-Wide Association Studies (GWAS), a substantial proportion of the identified risk-associated genes for prostate cancer are found within gene segments that are classified as intronic or non-coding. Traditionally, researchers encountered difficulties in efficiently identifying the transcriptional targets of risk alleles due to methodological limitations. However, with the advent of 3D genome mapping technology, it's now possible to identify the transcriptomic targets associated with prostate cancer risk alleles. The advancement of 3D genomics has enabled a further comprehension of the specific 3D structural shifts within the genome that underpin cancer development, and the long-range hormonal regulation of the cancer genome. By linking 3D genomics to prostate cancer research, we can not only deepen our understanding of prostate cancer pathogenesis but also potentially uncover novel diagnostic markers or therapeutic targets.



2. Three-dimensional structure of chromosomes

In 1953, the discovery of the double helix structure of DNA established a blueprint for understanding the preservation, replication, and transfer of genetic material [1]. The eukaryotic genome's physical blueprint is an intricate mesh of DNA and proteins, collectively termed 'chromatin.' This comprises genes, regulatory elements, and repetitive sequences that form the genetic content housed within chromosomes. Chromosomes, situated within the nuclear three-dimensional space, adhere to a structured organization. They maintain their defined structure and do not disperse. Instead, they fold into a hierarchical organization at various genomic scopes, resulting in distinct functional areas through efficient packing and structuring. This genomic organization ranges from larger to smaller scales, including hierarchical structures like Chromosome Territories (CT), bifurcated areas (A/B compartment), Topologically Associating Domains (TADs), and chromatin loops (RAD) [2].

Within the cellular nucleus during interphase, each chromosome occupies specific spatial regions commonly referred to as 'chromosome territories' [3]. The positioning of these territories exhibits a modicum of selectivity within the nucleus. Generally, gene-dense chromosomes are more likely to be situated in the nucleus's interior, whereas chromosomes with a lower gene density typically congregate towards the nuclear periphery. Chromosomal size also influences their positioning, with smaller chromosomes usually found closer to the nucleus's interior and larger ones near the nuclear rim [4]. Genome-wide interaction mapping has shown that interactions within a chromosome are more frequent than those between chromosomes, with interaction strength inversely related to spatial distance [5].

With the deployment of Hi-C technology, a checkerboard pattern of genomic contacts was observed. Open chromatin distinctly sets apart from closed chromatin leading to separate sectors. Individual chromosomes split into compartments labeled 'A' and 'B' resting upon the scale of gene expression activity [6]. More densely packed with genes, Compartment A showcases elevated translational manifestations and is home to a greater number of histone modifications that echo both active and dormant chromatin, when juxtaposed with Compartment B. Typically, Compartment A is located at the nucleus's center, while Compartment B predominates towards the nucleus's periphery [6]. Advances with in-situ Hi-C technology led to the discovery of numerous chromatin compartments, further classified into a minimum of five sub-compartment types, including A1-A2 and B1-B3[7].

In 2012, Professor Bing Ren's team conducted a study on the 3D structure of the genomes of human and mouse embryonic stem cells and differentiated cells. Upon visualizing the interaction matrix using a bin size smaller than 100 kb, apparent self-interacting "triangles" appeared on the heatmap. These regions were consequently termed Topologically Associated Domains (TADs). Each TAD is a relatively autonomous local unit, with the strength of interactions within a TAD significantly stronger than that observed between distinct TADs [8]. TAD boundaries are usually enriched with transcription start sites, adhesins, CTCFs, and markers indicating active modifications and housekeeping genes. These features may collectively contribute to the formation of TAD boundaries [7]. Disruption of TAD structures can lead to a series of developmental abnormalities and diseases [9].

Some regulatory elements in eukaryotic genomes, especially enhancers, frequently reside far from their corresponding genes in spatial terms. This spatial separation necessitates the formation of chromatin loops to facilitate regulatory processes [2]. Prominent types of chromatin loops include: promoter-enhancer loops, promoter-promoter loops, multi-comb protein-mediated loops, gene loops, and structural loops [10]. Chromatin loops have become a focal point of scientific study due to their crucial role in the localization of regulatory elements and target genes.

3. Unraveling the Three-Dimensional Structure of Chromatin: Techniques and Advances

Initial approaches to elucidate the complexities of chromatin's 3D structures relied on visualizing DNA sequences using techniques such as FISH and advanced microimaging [11]. Despite their

potential to provide understanding of gene activity regulation, their exactness and capacity fall short of expectations, thus posing challenges to the comprehensive detection and characterization of associations across arbitrary positions in the genome.

Building on this, Chromatin Conformation Capture (3C) assisted by high-throughput sequencing has been implemented and refined. In its earliest form, this technique dates back to 2002, the 3C method was created to gauge the intensity of pairwise interactions between two precise sites on *Saccharomyces cerevisiae* chromosomes. 3C and its derivatives operate by fixing cells in formaldehyde to facilitate chromatin cross-linking, followed by the utilization of restriction endonucleases for additional chromatin cross-linking. This process then employs restriction endonucleases (commonly HindIII and DpnII) to sever spatially adjacent DNA fragments before rejoining the sticky ends of these fragments. Sequencing methods are then used to procure the genome's interactions within the 3D nuclear space. As 3C could only detect pairwise interactions between two loci, the 4C technique was developed in 2006 to provide information about interactions between all loci with the locus of interest, yet it was incapable of identifying all potential site interactions.

Subsequently, the inception of the 5C technique, capturing interactions of multiple genome loci, represented an advance, but it remained restricted by the limitation of the target primer design, precluding a complete genome detection.

Hi-C technology's introduction in 2009 by Lieberman-Aiden's team revolutionized our ability to map interactions within chromatin on a comprehensive genomic range [6]. Later, significant improvements by Rao and his team in 2014 enriched this technology through the inception of in-situ Hi-C. This raised the detailing of the interaction matrix down to the 1kb mark, enhancing our visualization of the 3D panoramic view of chromatin and ameliorating signal detectability of Hi-C datasets [7]. Today, Hi-C stays as the go-to approach when dealing with chromatin conformation capture matters.

Moreover, an innovative set of procedures combined with ChIP and chromatin conformation capture methodologies were introduced. A prime example of this synthesis is ChIA-PET, which proposed capturing widespread chromatin interactions driven by a specific protein [12]. However, considerable limitations of ChIA-PET include a high cell requirement and vulnerability to errors, such as false positive results. To address these issues, Mumbach et al. pioneered the HiChIP process in 2016. As a refinement of in-situ Hi-C amalgamated with chromatin immunoprecipitation, HiChIP demands fewer cells and enhances detection accuracy at target loci. This improvement elevates the signal-to-noise ratio and hence has seen more extensive implementation compared to ChIA-PET [13].

4. Advances in three-dimensional genomics in prostate cancer research

Yuan and colleagues discovered that prostate cancer (PCa) risk genes were substantially enriched in Androgen Receptor (AR)-expressing cells through chromatin immunoprecipitation sequencing (ChIP-Seq) and chromatin interaction analyses. Analyzing the 3D chromatin interactions between risk loci and gene promoters, they found some risk alleles may participate in the transcriptional regulation of both proximate and distal genes in the primary tumor's linear genome, indicating that those genes are located in close proximity in 3D space. These findings imply that 3D genomic information can enhance our current methods of identifying transcriptional targets for PCa risk alleles [14].

Cancer, fundamentally, is linked to a series of changes at the molecular level within distinct cellular clumps within our bodies, ultimately resulting in uninhibited and aggressive growth. Our understanding of carcinogenesis has significantly accelerated due to the identification of pivotal genes heavily involved in controlling cellular progression and replication, forming the hotly debated frontlines of cancer research [15]. Over extensive periods of relentless research, major enablers and the inherent functional blueprint for carcinogenic growth, schematically outlining point changes and

subtle chromosomal transformations along the sequential genomic infrastructure that shape protein structures, have been charted. However, a new player has emerged—the 'epigenome,' articulating its ubiquitous influence on gene activity steered by transcriptional interactions. Expanding genomics research into the realm of three-dimensional architecture, distinctly impacted by various chromatin organizational levels, is fundamentally shifting our perspective on metastasis.

Numerous studies confirm the disturbance of the genome's three-dimensional configuration in a variety of cancers. The investigative work led by Taberlay and colleagues specifically isolates enigmas found within the 3D chromatin structure with particular focus on factors such as diverse copy counts, broad-spectrum epigenetic changes, and alterations in gene expression processes occurring in prostate cancer cases. It highlights the persistence of cancer cells to classify their genomes into TADs, albeit weakened by new domain boundaries. Evidence suggests an abundant influx of fresh boundaries specific to cancer structure appearing within sections presenting copy number discrepancies. Alterations in structural domains correspond to unique chromatin contacts within TADs that are cancer-specific and filled with regulatory elements, such as promoters and enhancers. This, in turn, influences the levels of gene expression [16].

The Androgen Receptor (AR)'s connection with prostate cancer is profoundly significant. Given that AR, once synchronized with testosterone, triggers the growth and multiplication of prostate cells, it emerges as a key target in prostate cancer treatment. With androgen levels directly affecting AR's function, the interaction between testosterone and AR results in AR activation and subsequent nuclear entry, thus controlling gene expression and cellular function. Non-coding RNAs, transcribed from distant regulatory regions, play a central role in regulating AR function, acting as critical scaffolding components to enhance AR-mediated long-range interactions, thereby influencing gene transcription and expression [17]. AR function is also regulated by co-activators and co-repressors, which bind to AR, and through chromatin remodeling, impact gene transcription.

Researchers have also analyzed the impact of three-dimensional genome disorders on gene expression by manipulating three-dimensional genome structure using CRISPR/Cas9 genome-editing technology. Guo et al. examined the Genome-Wide Association Studies (GWAS) risk loci involved in long-range interactions in prostate cancer. They found that when CTCF-anchored regions associated with prostate cancer risk were eliminated using CRISPR technology, gene expression within chromatin loops increased up to 100-fold [18]. This indicates that GWAS risk loci involved in long-range chromatin interactions can suppress gene expression within chromatin loops. These findings suggest that 3D genomics studies can unveil new insights into genetic predisposition to cancer.

5. The potential of three-dimensional genomics in prostate cancer therapy

The primary therapeutic strategies for prostate cancer, a hormone-sensitive malignancy, encompass surgical intervention, radiation therapy, cryotherapy, hormone therapy, chemotherapy, immunotherapy, and various targeted methodologies. Given the adaptive capabilities of cancerous cells, these interventions are typically deployed in a complementary manner along numerous pathways. Nevertheless, over time, the majority of cancers develop resistance to these interventions, underlying the necessity for more potent therapeutic strategies for hormone-responsive cancers. Considering the widespread alterations observed in the three-dimensional structure of most cancer genomes, topologically associating domains (TADs) present promising targets for novel therapies.

Approaches including but not limited to Hi-C and ChIA-PET have elevated our awareness regarding how variations at the genetic level within closely linked domain regions can potentiate the onset of cancer. By precisely altering or manipulating these TADs, researchers could potentially correct aberrant gene expression. CRISPR-based therapies hold great promise in this regard for directly targeting malfunctioning hormone receptors, significantly minimizing off-target risk. Furthermore, these CRISPR-based interventions can induce permanent modifications in the target receptor without

instigating drug resistance. Consequently, CRISPR-related methodologies provide more precise, and potentially more effective, treatment alternatives for hormonally mediated cancers.

One prospective therapeutic approach focuses on regulating the expression of tumor suppressors and oncogenes by modulating transcriptional regulatory elements such as the CCCTC-binding factor, or CTCF, more specifically. This can be achieved either through the removal or modification of CTCF as reported by Guo et al. or by manipulating DNA methylation at CTCF-associated sites which influences the boundaries of chromatin loops to employ the innovative CRISPR-Cas9 technology [18]. Such progress underscores the emerging potential of gene-modification tools like CRISPR-Cas9, paving the way for transformative developments in cancer therapy via the regulation of the three-dimensional genome.

6. Conclusion

The information synthesis derived from the studies propounds the fundamental role of three-dimensional genomics in elucidating the intricacies associated with prostate cancer. The rapidly evolving field, capitalized by advanced methodologies such as fluorescence in situ hybridization (FISH), chromosome conformation capture (3C) along with its derivations, Hi-C, in-situ Hi-C, and Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET), is monumentally enhancing our grasp on chromosomal architecture and its consequential impact on gene transcription and pathogenesis.

The implications of 3D genomics in the pathogenesis of prostate cancer have demystified various networks of interaction, especially where risk gene enrichment is witnessed around Androgen Receptor (AR) expressing cells. The breakthrough understanding that offers insight into the mechanisms by which distant risk alleles potentially influence the transcriptional regulation of proximate and distal genes within the primary tumor's genome has substantially broadened the scope of genomics research.

The introduction of CRISPR/Cas9 gene-editing technology has surfaced as a promising avenue in the genomics spectrum, offering the profound ability to meticulously target malfunctioning hormone receptors, thereby circumventing the risk of catalyzing drug resistance.

As we look toward the future, we envision the integrative utilization of these high-resolution methodologies stimulating unprecedented advances in cancer research and therapy management. By providing precision in characterizing the topologically associating domains (TADs) and subsequently understanding the mechanisms underpinning genomic alterations in cancers, novel opportunities are surfacing to devise treatment strategies tailored to fit individual patient profiles. Therefore, this insightful integration of 3D genomics into mainstream research extends an optimistic trajectory towards potentially more efficacious and targeted cancer treatment modalities.

References

- [1] Watson J D, Crick F H C. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid [J]. *Nature*, 1953, 171 (4356): 737 - 738.
- [2] Huang Q, Li Q, Zhang Y. Linking chromatin conformation to gene function [J]. *Hereditas*, 2020, 42 (01): 1 - 17.
- [3] Cremer T, Cremer M. Chromosome territories [J]. *Cold Spring Harbor perspectives in biology*, 2010, 2 (3): a003889.
- [4] Cremer T, Cremer C. Chromosome territories, nuclear architecture and gene regulation in mammalian cells [J]. *Nature reviews genetics*, 2001, 2 (4): 292 - 301.
- [5] Bolzer A, Kreth G, Solovei I, et al. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes [J]. *PLoS biology*, 2005, 3 (5): e157.
- [6] Lieberman-Aiden E, Van Berkum N L, Williams L, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome [J]. *science*, 2009, 326 (5950): 289 - 293.
- [7] Rao S S P, Huntley M H, Durand N C, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping [J]. *Cell*, 2014, 159 (7): 1665 - 1680.

- [8] Du Z, Zheng H, Huang B, et al. Allelic reprogramming of 3D chromatin architecture during early mammalian development [J]. *Nature*, 2017, 547 (7662): 232 - 235.
- [9] Tena J J, Santos-Pereira J M. Topologically associating domains and regulatory landscapes in development, evolution and disease [J]. *Frontiers in cell and developmental biology*, 2021, 9: 702787.
- [10] Bonev B, Cavalli G. Organization and function of the 3D genome [J]. *Nature Reviews Genetics*, 2016, 17 (11): 661 - 678.
- [11] Markenscoff-Papadimitriou E, Allen W E, Colquitt B M, et al. Enhancer interaction networks as a means for singular olfactory receptor expression [J]. *Cell*, 2014, 159 (3): 543 - 557.
- [12] Fullwood M J, Liu M H, Pan Y F, et al. An oestrogen-receptor- α -bound human chromatin interactome [J]. *Nature*, 2009, 462 (7269): 58 - 64.
- [13] Mumbach M R, Rubin A J, Flynn R A, et al. HiChIP: efficient and sensitive analysis of protein-directed genome architecture [J]. *Nature methods*, 2016, 13 (11): 919 - 922.
- [14] Yuan J, Houlahan K E, Ramanand S G, et al. Prostate Cancer transcriptomic regulation by the interplay of germline risk alleles, somatic mutations, and 3D genomic architecture [J]. *Cancer discovery*, 2022, 12 (12): 2838 - 2855.
- [15] Corces M R, Corces V G. The three-dimensional cancer genome [J]. *Current opinion in genetics & development*, 2016, 36: 1 - 7.
- [16] Taberlay P C, Achinger-Kawecka J, Lun A T L, et al. Three-dimensional disorganization of the cancer genome occurs coincident with long-range genetic and epigenetic alterations [J]. *Genome research*, 2016, 26 (6): 719 - 731.
- [17] Hsieh C L, Fei T, Chen Y, et al. Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation [J]. *Proceedings of the National Academy of Sciences*, 2014, 111 (20): 7319 - 7324.
- [18] Guo Y, Perez A A, Hazelett D J, et al. CRISPR-mediated deletion of prostate cancer risk-associated CTCF loop anchors identifies repressive chromatin loops [J]. *Genome biology*, 2018, 19: 1 - 17.