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Perspective

Chimeric RNAs and their implication in prostate cancer

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HIGHLIGHTS

- Chimeric ribonucleic acids (RNAs) form through gene fusion, trans-splicing, or cis-splicing between adjacent genes (cis-SAGe) mechanisms
- Chimeric RNAs influence prostate cancer (PCa) progression by acting as long noncoding RNAs (lncRNAs) or circular RNAs (circRNAs), coding fusion proteins, and misregulating parental genes
- Misregulated chimeric RNAs represent a novel repertoire for biomarkers and targets for PCa therapy

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Introduction

Specific gene fusions and their resultant fusion products, including chimeric ribonucleic acid (RNA) and protein, have long served as ideal tumor diagnostic markers and therapeutic targets. Despite this, only a few systematic studies on chimeric RNAs have been conducted in prostate cancer (PCa). In this study, we summarize the discovery pipeline, formation mechanisms, method of action, and future perspectives of chimeric RNAs. We aim to provide a viewpoint for exploring novel targets for diagnosing or treating PCa.

Pipeline for chimeric ribonucleic acid discovery, validation, and clinical application

Chimeric ribonucleic acids (RNAs) have gained significant attention owing to their broad implications in both cancer and normal physiology. To identify chimeric RNAs from RNA-sequencing (RNA-seq) data, over 40 prediction methods have been developed, such as JAFFA, SOAPfuse, ChimeraScan, and FuSeq. In a previous study,[1] we benchmarked 16 chimeric RNA prediction software and found that none of them were comprehensive. Combining software analysis is a good option to improve the sensitivity of chimeric RNA prediction. However, due to computational time and memory constraints, it is not feasible to combine all chimeric RNA prediction methods. Nonetheless, we investigated the best option of combining two software tools and found that certain combinations performed better than others (94.1%).[1] It is important to note that the performance of these software tools varies depending on the dataset and objects. Therefore, end users should choose a tool based on their specific needs.

After constructing a chimeric RNA library, experimental validation is crucial due to sequence homology and allowed mismatch errors in prediction methods. To confirm the chimeric RNA-specific junction sequence, it is recommended to use RNA samples from the same source as the RNA-seq analysis for reverse transcription-polymerase chain reaction (RT-PCR), and Sanger sequencing. Moreover, since clinical samples are heterogeneous, experimental validation on all types of cells from clinical tissue would be more meaningful in exploring the chimeric RNA expression. Studying the chimeric RNA in prostate cancer (PCa) cell lines alone is not comprehensive.

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We have developed a mature pipeline for chimeric RNA discovery and validation after years of work [Figure 1A]. However, to greatly reduce the cost of experimental verification, it is important to continue efforts to improve the specificity and sensitivity of software prediction.

Generating mechanism of chimeric ribonucleic acid

Chimeric RNAs are traditionally considered to be transcribed from gene fusions caused by chromosomal rearrangements.[2] Chromosomal rearrangements include insertions, deletions, and translocations that can result in cytogenetically distinct gene loci juxtaposed together. The most typical example is *TMPRSS2-ERG* (e1e4), which can be detected in about 50% of PCa cases and is reported to be the product of intra-chromosomal deletion or insertional gene rearrangement.[3] However, with the development of advanced deep sequencing technologies, novel types of non-canonical chimeric RNAs have been discovered to be generated from intergenic splicing without genomic aberrations.[2] These chimeric RNAs can be formed through trans-splicing or cis-splicing between adjacent genes (cis-SAGe) mechanisms. Trans-splicing is a splicing reaction between two pre-RNA molecules. The chimeric RNA uses the 5′ splice site from one gene and the 3′ splice site from another gene to splice together two exons from different parental genes. Our research team was probably the first to report the existence of trans-splicing between precursor messenger RNAs for *JAZF1* and *JJAZ1*, resulting in the formation of *JAZF1-JJAZ1* chimeric RNA through a physiologically regulated mechanism.[4] Our additional investigations provide evidence for the existence of trans-splicing in PCa, such as *SNX13-ATP2C1*.[3] The term "cis-SAGe" is preferred to distinguish these chimeric RNAs from trans-splicing events between neighboring genes. It refers to the intergenic splicing of directly adjacent genes with the same transcriptional orientation, resulting in read-through transcripts. In this case, RNA polymerase II neglects stop signals between neighboring genes, generating a read-through

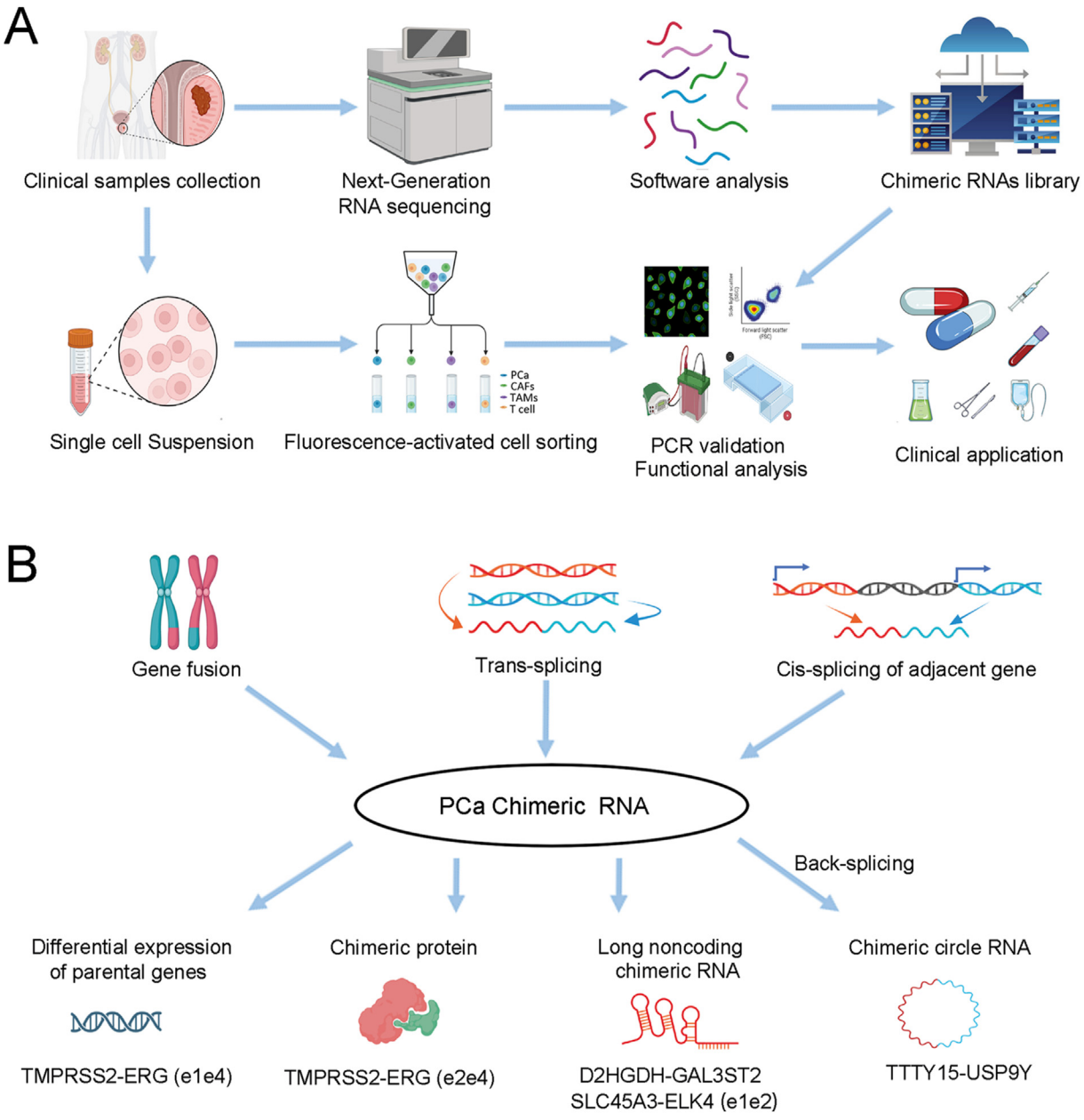

Figure 1. (A) Pipeline for chimeric ribonucleic acid discovery, validation, and clinical application. (B) Chimeric ribonucleic acid centric view. CAFs: Cancer-associated fibroblasts; PCa: Prostate cancer; PCR: Polymerase chain reaction; RNA: Ribonucleic acid; TAMs: Tumor-associated macrophages.

pre-transcript that is subsequently spliced into a mature chimeric RNA. Our research team was probably the first to report that *DUS4L-BCAP29*[5] and *SLC45A3-ELK4*[6] are both chimeric RNAs generated from cis-SAGe. We further generalized the rules under which cis-SAGe often occur: (1) 5′ gene and 3′ gene are multi-exonic, (2) 5′ gene is expressed, (3) 5′ gene and 3′ gene are close neighbors (<30,000 b), and (4) commonly, the second-to-last exon of the 5′ gene fuses with the second exon of the 3′ gene, resulting in the formation of chimeric RNAs by cis-SAGe.[7] These rules mainly govern the formation of chimeric RNA, as confirmed in current studies [Figure 1B]. However, it is important to note that the mechanisms of chimeric RNA formation may still be incompletely understood, and further research is needed to fully elucidate all possible mechanisms.

Mechanisms of chimeric ribonucleic acid function

Chimeric RNAs can impact tumor progression through various mechanisms [Figure 1B]. First, chimeric RNAs can affect PCa progression by misregulating parental gene expression. For example, in PCa, the promoter region of *TMPRSS2* becomes fused to the coding region of *ERG*, resulting in the formation of e1e4 *TMPRSS2-ERG*. As the promoter of *TMPRSS2* contains androgen-sensitive elements, it drives the overexpression of *ERG* in the presence of androgens, ultimately promoting the progression of PCa via pathways such as NO-cGMP, PI3KB-PKB/Akt, and Wnt.

Secondly, chimeric RNAs can expedite the neuroendocrine process of PCa by coding fusion proteins. *TMPRSS2-ERG* (e2e4), expressed higher in tumors, predicts poor prognosis in The Cancer Genome Atlas (TCGA), whereas its parental genes had no such association. Our recent study has demonstrated that compared to the most frequently detected *TMPRSS2-ERG* form (e1e4), *TMPRSS2-ERG* (e2e4) encodes additional 31 amino acids that could accelerate the neuroendocrine process of PCa by promoting the expression of neuroendocrine markers and facilitating chemotherapy resistance.[3]

Lastly, chimeric RNAs can also act as long noncoding RNAs (lncRNAs) or circular RNAs (circRNAs) in PCa.[2,8] For example, *SLC45A3-ELK4* is formed by a cis-SAGe mechanism and its expression was positively correlated with the malignancy of PCa. Additionally, neither parental gene expression showed such a correlation, suggesting that it is regulated differently from its parental gene expression. Although *SLC45A3-ELK4* has protein-coding potential, it mainly functions as a lncRNA in PCa.[6] We also discovered another chimeric RNA, *D2HGDH-GAL3ST2*, which is frequently detected in PCa, but not in normal cells or tissues. It mainly functions as lncRNA.[9] Interestingly, Vo et al. validated a chimeric circular *TTTY15-USP9Y* in the LNCaP cell line, suggesting that back-splicing could also occur after cis-SAGe.[8] Although they did not explore how *TTTY15-USP9Y* functions, this chimeric circular RNA in PCa may work similarly to other circRNAs as micro RNA sponges or by binding to proteins.

These three mechanisms have also been reported in other tumors, indicating that the modes of action of chimeric RNAs are universal.

Chimeric ribonucleic acids in prostrate cancer diagnosis and treatment

Intergenically spliced chimeric RNAs pose a challenge to the use of fusion RNAs in cancer detection and therapy. For example, many recently deposited chimeric RNAs in the Mitelman database (https://mitelmandatabase.isb-cgc.org/) are not specific to tumors. *DUS4L-BCAP29* was previously thought to be a biomarker for gastric cancer and PCa,[5] but our recent study revealed that *DUS4L-BCAP29* could also be detected in normal tissues and cells. While down-regulated *DUS4L-BCAP29* could inhibit the proliferation of RWPE-1 cells, overexpressed *DUS4L-BCAP29* could promote it. Despite this, *DUS4L-BCAP29* cannot be used as a diagnostic biomarker or therapeutic target.[5] Like *GAPDH*, a housekeeping gene, tumors and normal cells cannot tolerate the loss of *DUS4L-BCAP29*. Therefore, it is crucial that we move past these misunderstandings and recognize that chimeric RNAs that can impact cell proliferation and metastasis are cancer-specific, and can serve as biomarkers.

Conversely, intergenically spliced chimeric RNAs offer a novel repertoire for biomarkers and therapeutic targets. We identified and validated several chimeric RNAs in neuroendocrine prostate cancer (NEPC), including *TMPRSS2-ERG* (e2e4), *EEF2-SLC25A42*, *SNX13-ATP2C1*, and *FXYD2-DSCAML1,* all of which were specifically expressed in NEPC cells. Among these, *TMPRSS2-ERG* (e2e4) was expressed at a higher level in tumors and its expression predicted poor prognosis in the TCGA PCa study, while its parental genes had no such association, supporting the notion that this chimeric RNA could serve as a novel biomarker for NEPC. We also deep-mined chimeric RNAs from Cancer Cell Line Encyclopedia prostate RNA-seq dataset and characterized the landscape of chimeric RNAs in different PCa types, including hormone-sensitive PCa and castration resistance PCa.

In summary, chimeric RNAs expand the functional genome and offer insight into novel mechanisms of tumorigenesis, making them a valuable new addition to the transcriptome and a promising source for the development of biomarkers and therapeutic targets.

Future perspectives and conclusion

PCa is a common malignant tumor of the male genitourinary system with evident racial differences. Although the incidence of PCa is lower in the Asian population compared to the Western population, more than 70% of patients diagnosed with PCa for the first time are in the intermediate or advanced stage, making them more prone to metastasis and drug resistance. The 5-year survival rate is less than 30%, and the mortality-to-incidence rate ratio is much higher than that of the Western population (40% *vs.* 18%).[10] These facts suggest that racial differences may occur at the genomic, transcriptional, or even epigenetic level. On comparing chimeras' differences through deep analysis of TCGA (Western population) and Chinese Prostate Cancer Genome Epigenome Atlas (Chinese population), we found three times more chimeras in the Chinese population (data not shown), indicating that detecting these chimeric RNAs may have some diagnostic/prognostic value. Therefore, an in-depth exploration of chimeric RNAs will help identify specific biomarkers or treatment options for different populations.

It should be noted that not all the validated chimeras can be found in PCa cells due to the presence of interstitial cells. Increasingly, studies have demonstrated that interstitial cells, such as cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs), play a vital role in the progression of PCa. Therefore, chimeric RNAs may also participate in remodeling the tumor microenvironment. We propose that chimeric RNAs may regulate tumor progression in three aspects: (1) directly influencing tumor plasticity, (2) influencing the communications between tumor and microenvironment, and (3) regulating the tumor microenvironment.

In conclusion, misregulated chimeric RNAs represent a new repertoire for biomarkers and targets for therapy. Some chimeric RNAs, but not their parental genes, are correlated with PCa progression and/or are involved in the tumorigenesis of PCa. However, precautions need to be taken while assigning a chimeric RNA to be a biomarker/therapeutic target. Further studies on chimeric RNAs in different populations and in the tumor microenvironment are warranted to develop new therapeutic targets and biomarkers for PCa.

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Author contributions

Hui Li conceived and supervised the project and manuscript. Qiong Wang and Hui Li wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics statement

None.

Data availability statement

None.

Conflicts of interest

None.

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