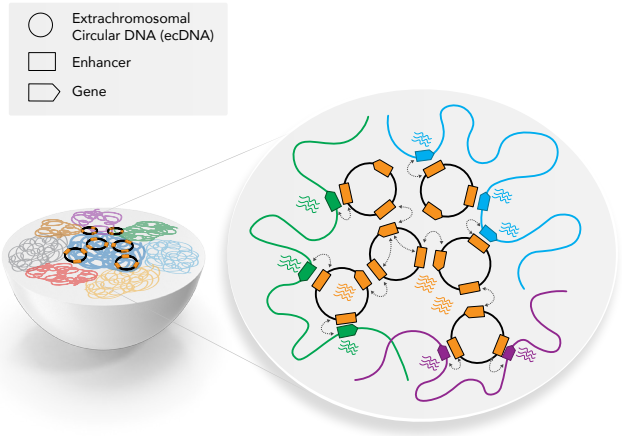
Detection and Characterization of Extrachromosomal DNA (ecDNA) in Medulloblastoma Using Arima-HiC+ Technology

Introduction

Extrachromosomal DNA (ecDNA) is a significant driver of aggressive tumor growth, promoting high oncogene copy number, intratumoral heterogeneity, accelerated evolution of drug resistance, and poor patient outcomes.[1,2,3]

This case study focuses on the application of Arima-HiC+ technology to detect and characterize ecDNA in medulloblastoma, the most common malignant pediatric brain tumor[4], highlighting its advantages and potential clinical implications.



Background

ecDNA consists of circular DNA molecules that exist outside the standard chromosomal DNA within cancer cells. These molecules are often present with several copies (e.g. amplified) and harbor oncogenes, contributing to rapid tumor growth and drug resistance.[2] Recent studies have identified ecDNA in various cancers, including medulloblastoma, and have linked its presence to poor prognosis.[4]

In medulloblastoma, ecDNA has been associated with specific molecular subgroups, such as SHH (Sonic Hedgehog), and is linked to enhanced oncogene activity through mechanisms like enhancer rewiring. Understanding and detecting ecDNA is crucial for developing targeted cancer treatments and improving patient outcomes (Figure 1).[4]

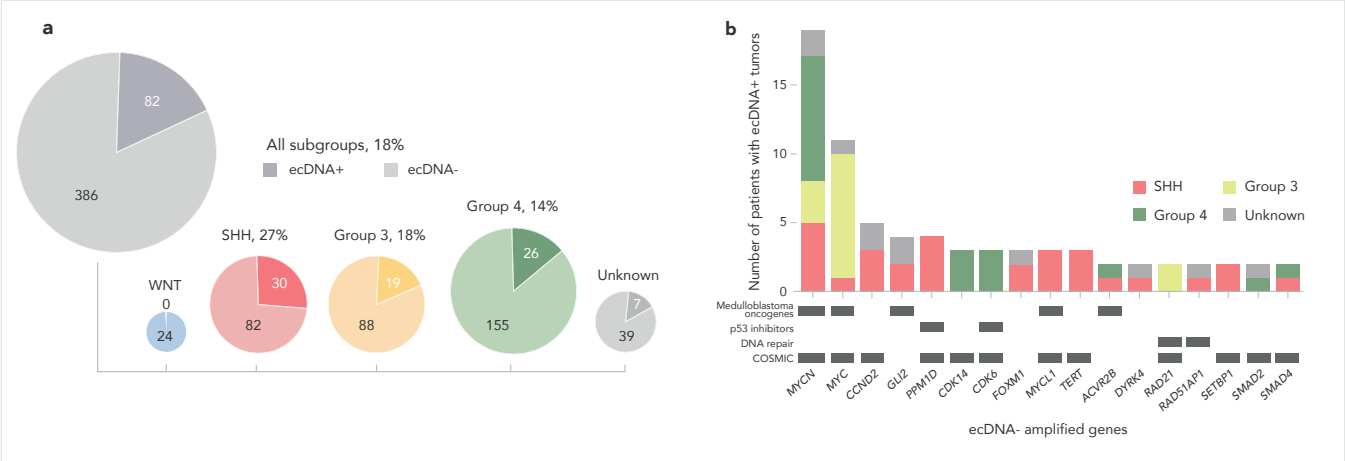


Figure 1. Prevalence of ecDNA in Medulloblastoma

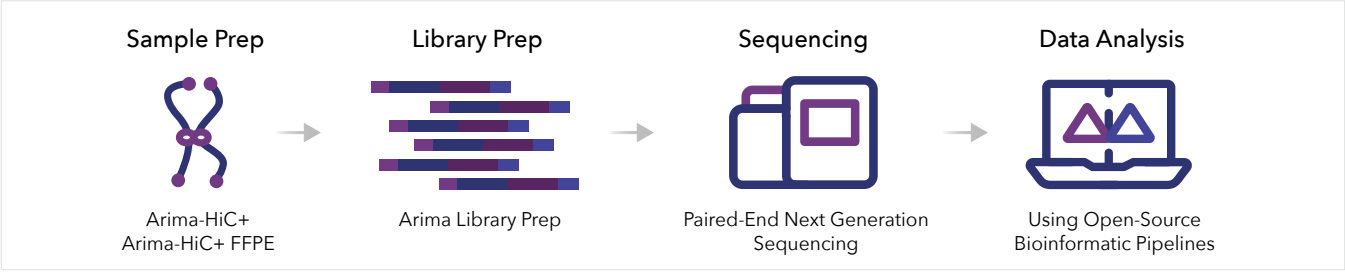


Figure 2. Arima Sample Preparation Workflow

Methods

Hi-C Sample Preparation

Clinical medulloblastoma tissue samples were processed using the Arima-HiC+ kit. The preparation involved crosslinking DNA, digesting with restriction enzymes, proximity ligation, and sequencing library preparation (Figure 2).[4]

Hi-C Sequencing and Data Analysis

Sequencing was performed on an Illumina NovaSeq platform. Hi-C reads were processed using Trimmomatic and aligned to the hg38 human genome reference with HiC-Pro. Visualization and contact normalization were performed with JuiceBox, and interactions were called using Juicer Tools GPU HiCCUPS. Interactions mapping to ecDNA were manually curated based on HiCCUPS interaction calls. Interchromosomal interactions were detected using FitHiC interchromosomal mode.[4]

Whole-Genome Sequencing and Analysis

Whole-genome sequencing (WGS) data were also analyzed to identify ecDNA. Data were preprocessed and aligned using internal pipelines, and Docker containers of fingerprint and AmpliconArchitect software were used for analysis on cloud genomics platforms like Cavatica and DNANexus. AmpliconArchitect and AmpliconClassifier tools were utilized to construct cyclic amplicons from WGS data, and Hi-C data validated the presence and structure of ecDNA.

Results

Detection of ecDNA in Medulloblastoma

Arima-HiC+ technology successfully identified ecDNA in medulloblastoma samples. The Hi-C maps revealed characteristic circular structures of ecDNA, distinguished by specific interaction patterns that deviate from linear chromosomal DNA (Figure 3).

In a cohort of 468 medulloblastoma patients, ecDNA was detected in 82 cases (18%), with the highest prevalence in the SHH subgroup (27%). Key oncogenes such as *MYC*, *MYCN*, and *CCND2* were frequently found on ecDNA. The presence of ecDNA was associated with significantly poorer outcomes, with patients twice as likely to relapse and three times as likely to die from the disease compared to those without ecDNA (Figure 1).

Enhancer Rewiring and Oncogene Activation

Hi-C data from ecDNA+ tumors revealed frequent enhancer rewiring events, where oncogenes on ecDNA formed new interactions with co-amplified enhancers. For instance, in a *MYC*-amplified Group 3 medulloblastoma sample, Hi-C analysis showed aberrant interactions between *MYC* and enhancers located on different chromosomes that were incorporated into the ecDNA, suggesting a mechanism for enhanced oncogene activation and tumor progression.

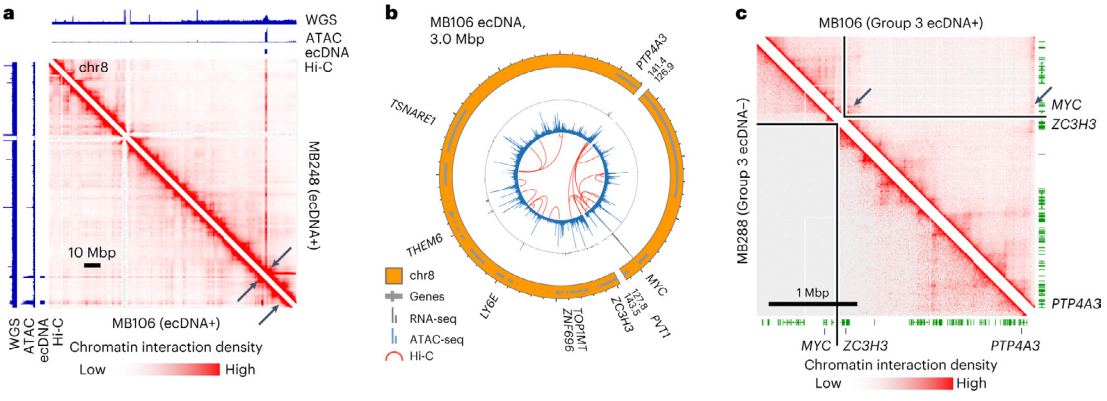


Figure 3. Visualization of ecDNA and Enhancer Rewiring.

Discussion

Advantages of Arima-HiC+ Technology

High Sensitivity and Characterization of ecDNAs: Because ecDNAs exhibit distinct structural patterns compared to chromosomal DNA, Arima-HiC+ technology provides a sensitive means for ecDNA detection. Furthermore, detailed insights into the 3D genome architecture of ecDNAs shed light on mechanisms of oncogene gene activation.

Versatility with Clinical Samples: The technology is compatible with various sample types, including **formalin-fixed paraffin-embedded (FFPE) tissues**, fresh frozen tissues and cell lines, ensuring broad applicability in clinical and research settings.

Visual Representation of ecDNA: Arima-HiC+ technology allows for the visual representation of ecDNA and enhancer rewiring events through heat map patterns, providing an intuitive and detailed view of the spatial organization and interaction dynamics of ecDNA, which aids in understanding its role in oncogenesis and tumor progression.

Detecting ecDNA using Arima-HiC+ technology can significantly impact cancer research by:

- **Identifying Oncogenic Drivers and Developing Targeted Therapies:** Understanding the role of ecDNA in oncogene amplification helps pinpoint crucial drivers of cancer progression, leading to potential therapeutic targets. Insights into ecDNA-mediated gene regulation can further guide the development of novel therapeutic strategies.
- **Comprehensive and Higher Resolution Genomic View:** Hi-C technology provides a genome-wide perspective on ecDNA interactions, offering more detailed information about ecDNA structure and interactions compared to traditional cytogenetic methods like fluorescence microscopy.
- **Improving Prognostic Models with Quantitative Analysis:** The integration of Hi-C with comprehensive bioinformatics tools enhances the accuracy and reliability of ecDNA detection. Hi-C data enables precise quantification of ecDNA interactions and copy numbers, improving prognostic models.
- **Dynamic Insights into ecDNA Behavior:** Hi-C captures the dynamic nature of ecDNA interactions, providing valuable information on their behavior during cell division and in response to treatments. This dynamic insight is crucial for understanding ecDNA's role in cancer progression and treatment resistance.

Conclusion

Arima-HiC+ technology offers a powerful tool for the detection and characterization of ecDNA in medulloblastoma. Its high-resolution mapping of 3D genome interactions and robust performance with challenging sample types make it an invaluable asset in cancer research and therapy development. By providing detailed insights into the structural dynamics of ecDNA, Arima-HiC+ technology enhances our understanding of cancer biology and supports the advancement of precision medicine in oncology.

References

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