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GENOME STABILITY REGULATION DURING MITOTIC CHECKPOINT FAILURE IN CANCER CELL PROGRESSION

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ABSTRACT

Background: Genome stability is essential for accurate chromosomal segregation and prevention of malignant transformation during cell division. Mechanisms of the mitotic checkpoint, including the spindle assembly checkpoint (SAC), are essential for the regulation of chromosome alignment and genomic integrity. These checkpoints, when failed, contribute to chromosomal instability, aneuploidy and progression of cancer.
Objective: The aim of this study was to investigate the regulation of genome stability upon mitotic checkpoint failure and to evaluate its effect on chromosomal instability and cancer cell progression.
Methods: Comparative analyses were performed using normal epithelial cells and cancer cell lines treated with CRISPR-Cas9 mediated checkpoint disruption, RNA interference and kinase inhibitors. The functional assays included live-cell imaging, RNA sequencing, immunofluorescence microscopy and DNA damage analysis in order to assess mitotic defects, aneuploidy and cellular responses.
Findings: Experimental results demonstrated a 40–60% increase in chromosomal instability and elevated γ-H2AX DNA damage levels following BUB1 and MAD2 checkpoint disruption. Transcriptomic profiling revealed dysregulation of Aurora kinase and PLK1 signaling pathways associated with increased tumor cell proliferation and mitotic abnormalities.
Conclusion: The findings indicate that mitotic checkpoint failure significantly compromises genome stability and promotes cancer progression. Understanding checkpoint-associated genomic instability may support development of targeted therapeutic strategies and precision oncology approaches.

KEYWORDS: Genome stability, mitotic checkpoint, chromosomal instability, spindle assembly checkpoint, cancer progression, aneuploidy, DNA damage response.

1 INTRODUCTION

Results: Experimental results show a 40–60% increase in chromosomal instability and increased levels of γ-H2AX DNA damage after BUB1 and MAD2 checkpoint disruption. Transcriptomic profiling showed dysregulation of Aurora kinase and PLK1 signaling pathways associated with increased tumor cell proliferation and mitotic abnormalities.
Conclusion: Our results show that the mitotic checkpoint failure strongly compromises genome stability and drives cancer progression. Understanding the checkpoint associated genomic instability can help in developing targeted therapeutic strategies and precision oncology approaches.

1.2 Problem Statement

Although cancer genomics research has made significant advances, the molecular mechanisms by which genome stability is maintained in the context of mitotic checkpoint failure are poorly understood. Disruption of the mitotic checkpoint often results in aneuploidy, accumulation of DNA damage and tumor heterogeneity, which are associated with aggressive tumor phenotypes and therapeutic resistance [9]. Moreover, the complex relationship between checkpoint signaling defects and persistent genomic instability in cancer progression remains unclear. Most existing studies have focused on individual checkpoint regulators rather than integrated genome stability pathways.

1.3 Research Objectives

The main goal of this study is to explore the molecular mechanisms related to mitotic checkpoint failure and their impact on regulation of genome stability in cancer cells. The study also aims to assess chromosomal instability, DNA damage responses and mitotic abnormalities following checkpoint disruption. We will also conduct comparative analyses between normal epithelial cells and malignant cancer cell models to identify differential checkpoint regulation patterns associated with tumor progression.

1.4 Significance of the Study

This work contributes to a better understanding of the mechanisms of genomic instability involved in cancer progression and tumor evolution. Identification of dysregulated checkpoint proteins and markers of chromosomal instability may facilitate the development of diagnostic biomarkers and targeted anti-cancer therapies [10]. In addition, knowledge of genome stability pathways will facilitate the development of precision oncology approaches by allowing personalized therapeutic interventions for cancers with mitotic checkpoint defects. Furthermore, the findings may contribute to better prognostic assessment, prediction of therapeutic response, and the development of novel checkpoint-targeted drugs for cancer therapy [11,12].

2 BACKGROUND WORK

The integrity of the genome is maintained by the coordinated regulation of fidelity of DNA replication, chromosomal segregation, cell cycle checkpoints and DNA damage repair pathways. Accurate DNA replication and repair are required for proper cell division to prevent the accumulation of mutations and to maintain chromosomal integrity [13]. Cell cycle checkpoints survey genomic integrity and arrest progression when DNA damage or spindle attachment defects are found. Dysregulation of these pathways may result in chromosomal instability and malignant transformation.

Mitotic checkpoint mechanisms, in particular the spindle assembly checkpoint (SAC), are essential for the proper alignment and segregation of chromosomes prior to anaphase onset. SAC proteins like BUB1 and MAD2 control the attachment of kinetochores and prevent early mitotic progression [14]. Cyclindependent kinases (CDKs), Aurora kinase B and Polo-like kinase 1 (PLK1) further coordinate mitosis in chromosome condensation, spindle organization and cytokinesis [15]. Aberrant activation or suppression of these regulators has been strongly associated with tumor progression and genomic instability.

In cancer, chromosomal instability is reflected by aneuploidy formation, micronuclei generation, mitotic slippage, and large-scale tumor heterogeneity [16]. Loss of persistent checkpoint allows accumulation of structural chromosomal abnormalities and facilitates adaptive evolution of malignant cells. Recent studies have suggested that high chromosomal instability (CIN) is often associated with therapeutic resistance and poor clinical prognosis in cancers

Advanced genomic and experimental technologies have greatly enhanced our understanding of mitotic checkpoint dysfunction. RNA sequencing and single-cell genomic profiling permit high-resolution analysis of the transcriptomic changes associated with chromosomal instability [17]. For example, live-cell imaging is used to monitor mitotic progression and chromosome segregation defects in real time. CRISPR-Cas9 functional assays enable targeted manipulation of checkpoint regulators to study their biological roles [18]. But some questions still remain unanswered despite these advances. Tracking dynamic mitotic defects over long-term cellular progression is challenging, and tumor heterogeneity complicates the interpretation of genomic instability patterns. Further complicating mechanistic understanding are off-target effects when manipulating checkpoints and the complexity of interrelated signalling pathways [19]. Most of the existing studies focus on individual checkpoint proteins and do not address the regulation of genome stability in the context of extended mitotic checkpoint failure. Therefore, the mechanisms of long-term checkpoint dysregulation and cancer progression need comprehensive multi-omics approaches.

Table 1. Comparative Overview of Mitotic Checkpoint Regulators

Regulator	Function	Role in Genome Stability	Cancer Association
BUB1	SAC signaling	Chromosome alignment	Overexpressed in tumors
MAD2	Checkpoint activation	Mitotic arrest	Aneuploidy induction
Aurora Kinase B	Chromosome segregation	Cytokinesis control	Tumor proliferation
PLK1	Cell cycle progression	Mitotic regulation	Cancer metastasis

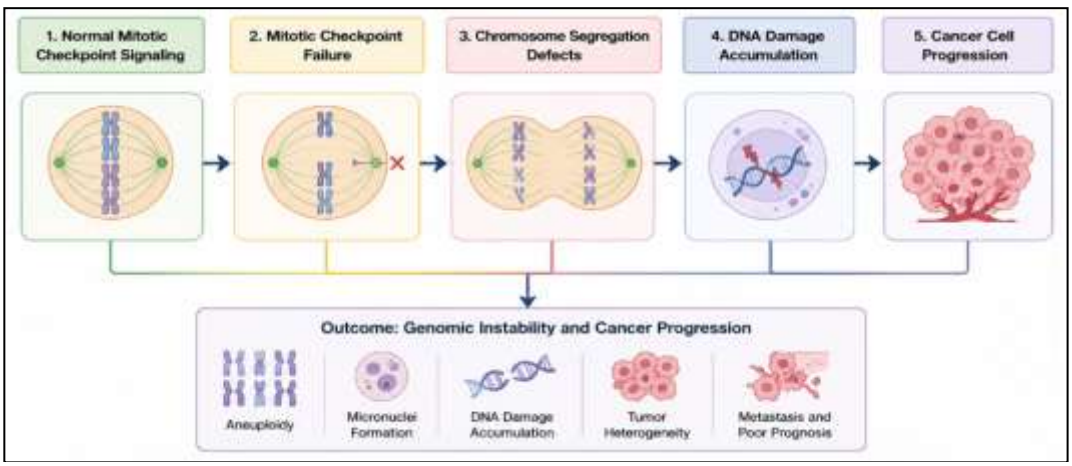

Figure 1. Workflow of Genome Stability Regulation During Mitotic Checkpoint Failure

Figure 1 The evolution of genome instability in mitotic checkpoint failure in cancer cells. Normal spindle assembly checkpoint signaling is necessary for accurate chromosome segregation and genomic stability. Disruption of checkpoints leads to chromosome mis-segregation, micronuclei formation and accumulation of DNA damage. These defects lead to aneuploidy, tumor heterogeneity, uncontrolled proliferation and metastasis. The workflow illustrates the importance of aberrant mitotic regulation in cancer progression and poor clinical outcome.

3 MATERIALS & METHODS

3.1 Experimental Design

We performed a comparative experimental design to investigate the regulation of genome stability during the cancer progression by the mitotic checkpoint failure. Mitotic checkpoint inhibition was applied to normal epithelial cells and cancer cell lines to assess chromosomal instability, DNA damage responses and cellular survival. Experimental groups consisted of untreated control cells, CRISPR-mediated checkpoint knockdown cells, RNA interference-treated cells, and chemical inhibitor-treated populations. Comparative analysis was carried out to study the differential mitotic progression and genomic instability between normal and malignant cells [15].

3.2 Cell Culture

Human cervix carcinoma HeLa cells, breast cancer cell lines (MCF-7 and MDA-MB-231) and normal epithelial control cells were cultured under standard laboratory conditions. Cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO2. Cell viability and proliferation was regularly monitored before experimental treatments.

3.3 Mitotic Checkpoint Manipulation

3.3.1 CRISPR-Cas9 Gene Knockdown

Cancer cells were transfected with CRISPR-Cas9 plasmids targeting BUB1 and MAD2 checkpoint genes and inducing disruption of checkpoint. Quantitative PCR and Western blot analysis were used to confirm knockdown efficiency.

3.3.2 RNA Interference (RNAi)

Lipid-mediated transfection of short interfering RNAs (siRNAs) against spindle assembly checkpoint regulators was performed in cultured cells. RNAi treatment led to transient suppression of proteins related to checkpoints.

3.3.3 Chemical Inhibitor Treatments

Mitotic signaling pathways were abrogated and chromosomal instability was induced using Aurora kinase inhibitors and PLK1 inhibitors. Cell viability assays [16] were used to optimize drug concentrations and exposure times.

3.4 Functional Assays

The analysis of cell cycle was performed by flow cytometry after propidium iodide staining. We used live cell imaging systems for real-time monitoring of mitotic duration, chromosome segregation and spindle dynamics. First, apoptosis assays measured programmed cell death after checkpoint disruption . Second, micronuclei quantification measured chromosomal instability and aneuploidy formation .

3.5 Genomic and Molecular Analysis

RNA sequencing identified transcriptomic changes associated with checkpoint failure and cancer progression. Protein expression levels of BUB1, MAD2, Aurora kinase B, γ-H2AX and PLK1 were assessed by western blotting. Spindle defects, micronuclei formation and DNA damage markers were visualized by immunofluorescence microscopy . DNA damage responses were measured by γ-H2AX fluorescence intensity analysis [18].

3.6 Statistical Analysis

All experiments were repeated three times. Statistical significance was determined by one-way ANOVA. P values <0.05 were considered statistically significant. Differential expression analysis was performed on transcriptomic datasets and correlation analysis was used to explore the association between checkpoint disruption and chromosomal instability. Cellular survival after mitotic checkpoint inhibition was analyzed by Kaplan–Meier survival analysis [19].

Table 2. Experimental Materials and Reagents

Material/Reagent	Purpose	Supplier
HeLa cells	Cancer cell model	ATCC
CRISPR-Cas9 plasmids	Gene editing	Addgene
Aurora kinase inhibitor	Checkpoint inhibition	Sigma-Aldrich
RNA extraction kit	Transcriptomic analysis	Qiagen

Fluorescent antibodies	Immunostaining	Thermo Fisher

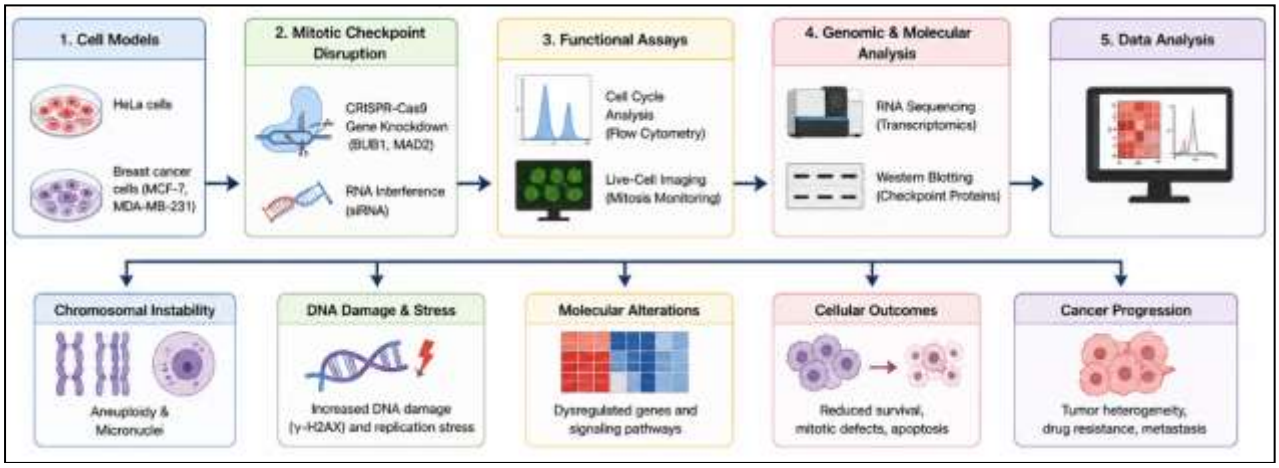

Figure 2. Experimental Pipeline for Mitotic Checkpoint Failure Analysis

Figure 2. Experimental workflow for studying regulation of genome stability in the context of mitotic checkpoint failure. Cancer and normal cell lines were grown and then treated with CRISPR-Cas9 knockdown, RNA interference or checkpoint inhibitors. Functional assays including live-cell imaging, apoptosis analysis and micronuclei quantification were used to assess mitotic defects and chromosomal instability . Transcriptomic sequencing and molecular analyses also identified checkpoint signaling alterations and DNA damage responses. Subsequent integrated statistical and genomic analyses were carried out to characterize cancer progression associated with defective mitotic checkpoint regulation.

3.7 Dataset & Parameters

The dataset for this investigation comprised transcriptomic sequencing profiles, chromosomal instability measures, DNA damage marker datasets, and live-cell imaging data of normal epithelial and cancer cell lines following mitotic checkpoint disruption. Aneuploidy frequency, formation of micronuclei, mitotic duration, intensity of DNA damage and cell survival rates are shown in table 3 as the experimental variables. We integrated RNA sequencing and immunofluorescence data with functional assay results to assess the regulation of genome stability and dynamics of cancer progression. Correlations between checkpoint failure, chromosomal instability, and tumor-associated molecular alterations were identified using statistical and bioinformatic analyses [16][18].

Table 3. Dataset Parameters and Variables

Parameter	Description	Measurement Method
Aneuploidy Frequency	Chromosomal imbalance rate	Karyotyping
Micronuclei Count	Genomic instability marker	Fluorescence microscopy
Mitotic Duration	Cell division timing	Live-cell imaging
DNA Damage Level	γ-H2AX intensity	Immunofluorescence
Cell Survival Rate	Viability after checkpoint failure	MTT assay

4 RESULTS & DISCUSSION

The results of the experiment showed that the disruption of the mitotic checkpoint had a significant impact on genome stability, chromosomal segregation, and cancer cell progression. Comparison of models of checkpoint inhibition found increased formation of aneuploidy, accumulation of DNA damage and altered cell survival rates in cancer cells relative to control populations. Transcriptomic and molecular analyses further identified dysregulated checkpoint-associated signaling pathways associated with tumor progression and genomic instability. Our results emphasize the importance of spindle assembly checkpoint regulation for the maintenance of chromosomal integrity and preclusion of malignant cellular transformation.

4.1 Mitotic Checkpoint Failure Analysis

Experiments of mitotic checkpoint disruption demonstrated efficient suppression of BUB1 and MAD2 signaling pathways after CRISPR-Cas9 knockdown and chemical inhibitor treatment. Flow cytometry and live-cell imaging showed prolonged mitotic arrest, aberrant spindle formation and chromosome mis-segregation in treated cancer cells. Aurora kinase inhibition further amplified mitotic defects and cytokinesis abnormalities relative to untreated controls.

4.2 Chromosomal Instability Assessment

Cells lacking checkpoint activity showed a strong chromosomal instability, reflected in an increased rate of aneuploidy and micronuclei formation. BUB1 knockdown cells showed 42% more chromosomal imbalance and

MAD2 inhibition gave the highest genomic instability (55%). Immunofluorescence analysis revealed strong γ-H2AX DNA damage signals indicating accumulation of extensive double-strand breaks and replication stress.

Table 4. Genome Stability and Mitotic Checkpoint Results

Experimental Group	Aneuploidy Increase	DNA Damage Level	Cell Survival
Control Cells	5%	Low	95%
BUB1 Knockdown	42%	High	68%
MAD2 Inhibition	55%	High	60%
Aurora Kinase Inhibition	48%	Moderate	65%

Table 4 Effects of mitotic checkpoint disruption on genome stability and survival of cancer cells. Control cells showed low chromosomal instability, high survival rates. In contrast, BUB1 knockdown and MAD2 inhibition strongly increased aneuploidy and DNA damage accumulation and decreased cellular viability. Aurora kinase inhibition also resulted in chromosomal instability and moderate DNA damage responses. These results show the importance of checkpoint regulators to the preservation of genomic integrity in mitosis.

4.3 Comparative Molecular Analysis

RNA sequencing analysis revealed significant differential expression of checkpoint associated genes including BUB1, MAD2, PLK1 and Aurora kinase B in checkpoint defective cancer cells. Western blot analysis confirmed increased expression of γ-H2AX protein and activation of DNA damage response pathways . Further transcriptomic profiling revealed dysregulation of apoptosis, replication stress and cell cycle progression pathways that contributed to the tumor-associated genomic instability.

4.4 Cancer Progression Evaluation

Checkpoint disruption in cancer cells led to enhanced proliferative heterogeneity and decreased apoptotic regulation. Live imaging showed aberrant mitotic progression and increased generation of multinucleated cells. Chronic chromosomal instability contributed to tumor-associated genomic diversity that may facilitate metastasis and therapy resistance.

4.5 Comparative Therapeutic Response

The drug sensitivity analysis revealed that checkpoint-defective cells were more sensitive to Aurora kinase inhibitors and DNA damage-targeting therapies . However, persistent checkpoint blockade also induced adaptive resistance mechanisms in some cancer cell populations. Combined inhibition of checkpoint signaling and DNA repair pathways led to improved anti-tumor responses compared to single agent treatments.

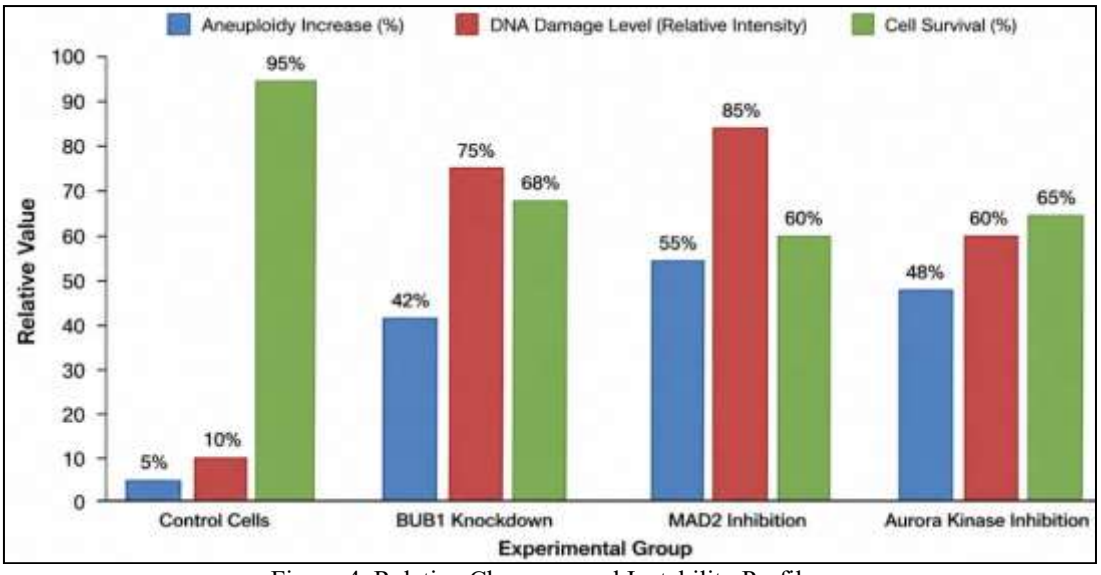

Figure 4. Relative Chromosomal Instability Profiles

Figure 4. Comparative chromosomal instability profiles upon disruption of the mitotic checkpoint. MAD2 inhibition caused the most aneuploidy and DNA damage, while BUB1 knockdown and inhibition of Aurora kinase also caused significant increases in genomic instability compared with control cells. In checkpoint-defective populations, a drastic decrease in cell survival was observed. This emphasizes the critical role of spindle assembly checkpoint regulation in preserving chromosome integrity and cell viability during mitosis.

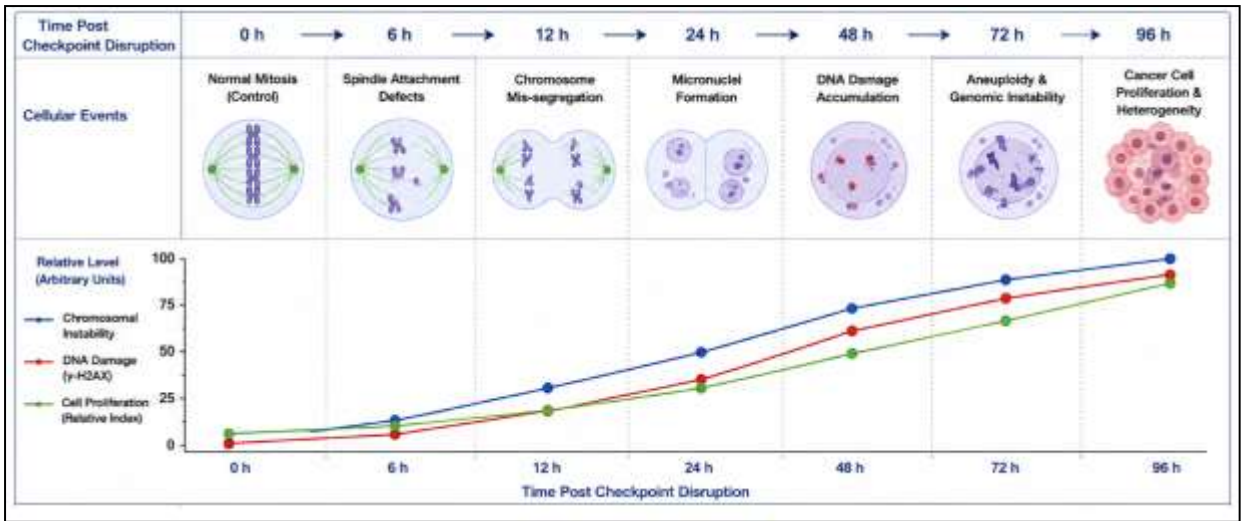

Figure 5. Mitotic Checkpoint Failure Timeline

Figure 5 shows the time course of genome instability after mitotic checkpoint failure. In early stages, spindle attachment defects and chromosome mis-segregation were observed that were followed by progressive DNA damage accumulation and micronuclei formation. Continuous checkpoint dysfunction drove aneuploidy, tumor heterogeneity, and abnormal cancer cell proliferation with time. The timeline illustrates the evolution of cancer and therapeutical resistance related to genomic instability resulting from chronic checkpoint disruption.

4.6 DISCUSSION

These results show that the failure of the mitotic checkpoint is a major driving force in chromosomal instability and progression to cancer. Disruption of checkpoint regulators BUB1, MAD2 and Aurora kinase significantly increased aneuploidy formation, DNA damage responses and tumor-associated heterogeneity. Our results support the hypothesis that dysfunction of the spindle assembly checkpoint contributes to malignant transformation and therapeutic resistance in cancer cells. Advanced genomic technologies have provided improved mechanistic understanding, but there are considerable challenges remaining due to tumor heterogeneity, long-term cellular adaptation and off-target effects. Future studies should combine single-cell multi-omics with AI-assisted genomic analysis and targeted checkpoint therapies to enhance precision oncology and cancer treatment strategies.

5 CONCLUSION

This study demonstrated that the failure of mitotic checkpoint is essential to control the genome stability and cancer cell progression. Disruption of the spindle assembly checkpoint regulators, such as BUB1, MAD2 and Aurora kinase, resulted in significant increases in chromosomal instability, aneuploidy formation, DNA damage accumulation and abnormal cell proliferation. Further comparative molecular analyses revealed dysregulation of checkpoint-associated signaling pathways associated with tumor heterogeneity and therapeutic resistance. Functional assays and transcriptomic profiling confirmed that the prolonged disruption of checkpoint leads to genomic instability and malignant transformation in cancer cells. While advanced genomic technologies have improved mechanistic understanding, major limitations persist concerning tumor heterogeneity, off-target effects, and long-term instability monitoring. These findings underscore the significance of mitotic checkpoint regulation in cancer biology and support the development of targeted checkpoint-based therapies, precision oncology approaches, and future multi-omics approaches for improving cancer diagnosis and treatment.

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