Unravelling the complexities of chromosomal instability in cancer: exploring molecular pathways and potential therapeutic targets
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CHAPTER 1

General Introduction and Thesis Outline
General Introduction

Chromosomal Instability and Tumour Progression

Chromosomal instability (CIN) is a hallmark of cancer and is intricately linked to tumour progression due to the accumulation of chromosomal abnormalities over time (D’Assoro et al., 2010). The association of CIN with various cancers, such as colorectal, triple-negative breast cancer (TNBC), gastric, and hepatocellular carcinoma, underscores its crucial role in cancer development and progression (Ye et al., 2020; Paulson et al., 2009; Hoevenaar et al., 2020). The collective findings of these studies emphasise the substantial influence of CIN on cancer development, affirming its fundamental role in tumour formation. Recent insights highlight the critical role of CIN in metastatic transformation, emphasising its significant influence on cancer progression (Gao et al., 2016).

TNBC, known for its aggressive nature and lack of hormone receptors and HER2 amplification, is significantly affected by CIN. This contributes to the rapid growth of the disease, limited treatment options, and poor prognosis (Collignon et al., 2016; Shuaib et al., 2022). The role of CIN in TNBC is linked to cancer initiation, invasion, and chemotherapy resistance, particularly to taxanes (Colón-Marrero et al., 2021; Meyer et al., 2020).

Recent studies have enhanced our understanding of CIN in TNBC, revealing new treatment options. Research indicates that intermediate levels of CIN can modulate drug sensitivity, offering opportunities to enhance treatment efficacy (Vargas-Rondón et al., 2020). The Triple Negative Breast Cancer Trial (TNT trial) (NCT00532727) suggests that TNBC patients with intermediate CIN levels, particularly those without high-level amplifications (HLAMP), may derive more significant benefit from carboplatin over docetaxel, illustrating the intricate relationship between genomic instability and therapeutic response (Sipos et al., 2021). Furthermore, studies demonstrate that transient CIN can hasten the development of resistance to cancer treatments, emphasizing the need for therapeutic strategies that exploit CIN-induced phenotypic plasticity (Lukow et al., 2021). Intriguingly, the efficacy of paclitaxel in TNBC is attributed not to mitotic arrest but to the induction of multipolar mitotic spindles, with pre-existing CIN levels enhancing drug sensitivity (Scribano et al., 2021), 2021). These positions baseline CIN levels as a potential biomarker for predicting paclitaxel response, suggesting that increasing CIN or maintaining multipolar mitosis could improve the clinical utility of paclitaxel in managing breast cancer (Scribano et al., 2021).

Moreover, incorporating CIN-targeted strategies into combination therapies, such as immunotherapy and targeted agents, shows promise, potentially leading to enhanced
therapeutic outcomes (Clark & Yang, 2021). For instance, combining checkpoint inhibitors with agents that worsen CIN could synergistically boost tumour immunogenicity and make TNBC cells more responsive to immunotherapy (Castellanos et al., 2023; Hunia et al., 2022; Rieckhoff et al., 2020).

In conclusion, CIN is a hallmark feature of breast cancer, including TNBC, and also plays an important role in determining treatment strategies and patient outcomes. The ongoing exploration of CIN’s mechanisms and its interaction with various therapeutic approaches is crucial for developing more advanced and effective cancer treatments.

CIN and the Tumour Microenvironment

The tumour microenvironment (TME) plays a crucial role in cancer progression, and involves a complex network of cells, extracellular components, and signalling molecules (Zhang et al., 2020; Baghban et al., 2020; Neophytou et al., 2021). The TME, which includes immune cells, stromal cells, the extracellular matrix (ECM), blood vessels, and signalling molecules, has a significant impact on tumour growth, invasion, and metastasis (Elsawa et al., 2011; Neophytou et al., 2021; Patel et al., 2018).

The interactions within the TME, particularly between tumour cells and the immune system, are receiving significant attention (Bakhoum et al., 2018; Baghban et al., 2020). To date, immunotherapy is an effective treatment in many patients (Yang & Zhang, 2020). However, because it is not effective as we expect in a significant group patient, better biomarkers of success for treatment are needed. Cytokines play a crucial role in shaping the interactions between tumours and the immune microenvironment, influencing overall cancer progression (Elsawa et al., 2011). The presence of immune checkpoint molecules like PD-L1/PD-1 and CTLA-4, along with ectonucleotidases such as CD73 and CD39 within the TME, underscores the complex immune evasion strategies employed by cancer cells (Porcellato et al., 2021; Goueli & Hsiao, 2019).

An emerging area of interest in cancer research is the link between CIN and the TME. Mitotic errors induced by CIN trigger a cascade of transformations within the TME, including alterations in immune cell makeup, restructuring of the extracellular matrix (ECM), modifications in extracellular vesicle interactions, alterations in angiogenesis-promoting factors, and changes in the communication between tumour and stromal cells (Cao et al., 2020; Lima et al., 2021; Lu et al., 2022; Dai et al., 2018). These combined changes contribute to creating a TME that supports tumour expansion and spread. Recent studies have highlighted the crucial role of CIN in facilitating interactions between tumours and the
microenvironment, especially through the cGAS-STING pathway (Bakhoum et al., 2018; Li et al., 2021; Basit et al., 2020; Tripathi et al., 2022). This relationship highlights the significant impact of CIN on both cancer cells and the entire TME (Hong et al., 2022; Bakhoum et al., 2018; Bakhoum & Cantley, 2018). However, aneuploid tumours may evade immune detection by altering Stat1 signalling and Myc activity (Schubert et al., 2021). This might reveal a new therapeutic angle: modulating the immune landscape in aneuploid cancers to improve immune system recognition and attack, offering a promising strategy for treatment (Schubert et al., 2021). Further work is required to distinguish the specific responses to CIN among the various cellular and acellular components within the TME.

The interaction between CIN and the TME encompasses various aspects of cancer biology, including inflammation, immune interactions, and the dynamic exchange between genetic instability and the surrounding microenvironment. Enhancing our understanding of these interactions offers promising opportunities for new therapeutic strategies.

**SAC Inhibition and Cancer Treatment**

The Spindle Assembly Checkpoint (SAC) is a crucial regulatory mechanism that ensures accurate chromosome segregation during mitosis, playing a key role in maintaining genomic stability (Lara-Gonzalez et al., 2021). Disruptions in the SAC functionality increase the rate of CIN.

Recent research has emphasized the potential of targeting the SAC as a therapeutic approach in cancers with CIN. Inhibiting SAC components, which causes chromosome missegregation, can lead to cell death, especially in aneuploid or CIN-afflicted cell lines and tumours (Cohen-Sharir et al., 2021; Libouban et al., 2017). This strategy is supported by evidence indicating that many tumours increase the expression of SAC components, such as Monopolar spindle 1 (MPS1), to maintain survivable levels of CIN, making it a potential target for cancer therapy (Slee et al., 2014). Furthermore, disrupting SAC-mediated phosphorylation events, such as those involving DAB2IP, could destabilize the mitotic checkpoint complex and increase the rates of chromosome missegregation, highlighting the therapeutic potential of SAC components (Yu et al., 2022).

The inhibition of SAC holds the potential for selectively targeting cancer cells with strong dividing ability. By bypassing the checkpoint, SAC inhibitors can disrupt cytokinesis, leading to cells with multiple nuclei and significantly hindering cancer progression (Colombo et al., 2010; Tardif et al., 2011). Selective inhibitors such as NMS-P715 and MPI-0479605 have demonstrated efficacy in inducing aneuploidy and subsequent cell death in
cancerous cells while sparing normal cells, attributed to their differential MPS1 expression levels (Colombo et al., 2010; Tardif et al., 2011). This strategy, however, may also cause some genomic instability in non-cancer cells, highlighting the need for careful application of SAC inhibitors to ensure cancer specificity while minimizing side effects.

The intricate connection between SAC inhibition and CIN presents a challenge in cancer treatment. While increasing CIN rates through SAC inhibition can impede tumour growth and potentially limit metastasis, it also increases CIN rates in normal dividing cells, which will have detrimental effects on healthy tissue in the short term, but also poses a risk of new transforming events in the future (Zasadil et al., 2016).

Ongoing clinical trials are examining the effectiveness of SAC inhibitors in solid tumours, but identifying reliable predictive biomarkers for effective use of these inhibitors remains a significant challenge. The identification of these biomarkers would significantly improve the precision and effectiveness of SAC-targeted therapies, allowing for personalized treatment strategies that could greatly enhance patient outcomes.

The investigations aim to explore the correlation between SAC inhibition and CIN to discover novel therapeutic opportunities by exploiting the weaknesses of cancer cells that rely on SAC for their abnormal growth. By optimizing the utilization of SAC inhibitors and potentially combining them with other therapeutic approaches, there is a promising opportunity to advance innovative cancer therapies that leverage the harmful effects of CIN in tumour cells, with the goal of significantly enhancing therapeutic efficacy.

**Extracellular Vesicles as Cancer Treatment Source**

Extracellular vesicles (EVs) are gaining significant attention in oncological research due to their potential across various cancer types, including their promising applications in the challenging realm of TNBC (Dong et al., 2022; Yi, 2023). These comprehensive studies clarify the different roles of EVs in cancer biology, including their impact on disease progression, precise diagnosis, and therapeutic interventions, with a specific focus on TNBC (Dong et al., 2022; Yi, 2023).

An intriguing avenue of research is exploring the use of macrophage-derived EVs as vehicles for targeted drug delivery, particularly in the context of TNBC (Haney et al., 2020). These EVs, distinct from their cancer-derived counterparts, hold the potential for precise TNBC targeting and efficient delivery of anticancer agents, showcasing notable efficacy in preclinical models. This distinction underscores their utility as therapeutic vehicles, offering
a nuanced approach to influencing TNBC progression (Haney et al, 2020). This distinction highlights their potential as therapeutic tools rather than contributors to TME dynamics. Further studies have enhanced our understanding of the significant role of EVs in shaping the pathological landscape of TNBC (Senigagliesi et al, 2022; Desai et al, 2022). Recent studies have shown that EVs promote tumour growth, spread, and drug resistance, significantly impacting the aggressive nature and treatment resilience of TNBC (Senigagliesi et al, 2022; Desai et al, 2022). This highlights the significance of EVs in influencing the interaction between cancer cells and their microenvironment, which contributes to the well-known treatment difficulties of TNBC.

The diverse molecular cargos transported by EVs, including proteins, lipids, mRNAs, non-coding RNAs, and DNA fragments, play a crucial role in influencing the tumour microenvironment (Xu et al, 2022; Bazzan et al, 2021; van Niel et al, 2018). Intriguingly, the role of Annexin A2 (AnxA2) in tumour-derived EVs has been identified as a key factor in establishing metastatic niches, providing new insights into the mechanisms of TNBC progression and metastasis (Desai et al, 2022). Moreover, the correlation of specific EV-associated cytokines, such as EV_APRIL, EV_CXCL13, and EV_VEGF-A, with poorer survival outcomes in TNBC patients undergoing chemotherapy, highlights the potential of EVs as prognostic biomarkers and therapeutic targets (Jung et al, 2023).

EV-mediated modulation of the TME is crucial in promoting the invasive and metastatic capabilities of TNBC, highlighting the potential of EVs as therapeutic targets and tools (Kumar et al, 2024; Senigagliesi et al, 2022). The identification of specific targets within the EVs landscape, such as ROCK1, EGFR, and CD47, paves the way for new combination therapies aimed at disrupting multiple tumorigenic pathways simultaneously, thereby improving the effectiveness of treatments for triple-negative breast cancer (Si et al, 2022).

In conclusion, the exploration of EVs in the context of TNBC highlights ongoing efforts to understand and combat this aggressive form of breast cancer. The diverse approaches and targets being studied demonstrate the complexity of TNBC but also highlight the potential for developing innovative and more effective therapeutic strategies. This includes leveraging the unique properties of EVs to address the challenges posed by this hard-to-treat cancer subtype.
Aim of Study

This thesis provides a thorough investigation of the relationship between CIN, SAC inhibition, EVs, and their impact on cancer progression, specifically focusing on TNBC. The study aims to dissect the intricate dynamics of CIN and SAC inhibition in TNBC, with a particular focus on the molecular roles of HDAC4, CDC20, and EFEMP1. We explore HDAC4’s influence on cell cycle regulation and tumour growth, CDC20’s contribution to SAC inhibitor sensitivity, and EFEMP1’s role in mediating tumour metastasis through EVs. Our work contributes to a better understanding of TNBC’s aggressive nature and identifies potential therapeutic interventions tailored to combat this challenging cancer subtype effectively.
Thesis Outline

Chapter 1: General Introduction and Thesis Outline

Chapter 2: CIN and Tumour Microenvironment Dynamics

In Chapter 2, we critically examine the role of CIN in tumour recurrence, metastasis, and therapy resistance, particularly its interaction with the tumour microenvironment in various cancers. We evaluate how targeting CIN affects the tumour microenvironment to potentially improve treatment outcome. We conclude how targeting CIN could potentially refine treatment outcomes by altering the TME, dissecting pathways like the cGAS-STING signalling and the implications of epithelial-mesenchymal transition (EMT) alongside metabolic signalling and TME remodelling. This chapter underscores the importance of the CIN-TME nexus in developing effective CIN+ cancer therapies, proposing targeted strategies such as immune landscape modulation, exploiting metabolic vulnerabilities, and manipulating CIN's downstream effects to pave the way for innovative treatments that leverage a deeper understanding of CIN's role in cancer progression.

Chapter 3: Targeting MELK overexpression in cancer therapeutics

In Chapter 3, we explore the effects of maternal embryonic leucine zipper kinase (MELK) overexpression in cancer and propose a new approach to cancer treatment by targeting HDAC4. We investigate the role of MELK in cancer, particularly its overexpression and the subsequent impact on tumour growth, chemotherapy resistance, and recurrence. Through a comprehensive high-throughput genetic screen in yeast, we identify critical targets, notably LAG2 and HDA3, which sensitisce cells to increased MELK activity, unveiling a strategic therapeutic target—HDAC4, HDA3's human analogue. This approach suggests that inhibiting HDAC4 in MELK-overexpressing cancers could provide a focused strategy to combat tumour progression, offering insights into potential therapeutic interventions and highlighting the importance of the MELK-HDAC4 interaction in developing targeted cancer treatments.

Chapter 4: The role of CDC20 in cancer and SAC inhibitor sensitivity

In Chapter 4, we present and identify a novel function of CDC20 in modulating sensitivity to SAC inhibitors through a genome-wide CRISPR-Cas9 screen in NIH-3T3 mouse fibroblasts. Our investigation aimed to identify genes whose absence promotes cell proliferation under SAC inhibition, with a particular focus on the Mps1 inhibitor reversine. Our work reveals a strong correlation between CDC20 expression and the cellular
response to SAC inhibitors. CDC20, a crucial activator of the anaphase-promoting complex (APC/C) during mitosis, was identified as a key gene whose reduction leads to enhanced resistance against SAC inhibition, positioning it as a potential biomarker for SAC inhibitor-based treatments. Subsequent validation experiments, involving CDC20 knockdown and knockout, reinforced its critical role, as CDC20-depleted cells exhibited reduced sensitivity to reversine. We identify a link between CDC20 expression levels and cellular responses to SAC inhibition, which could have implications for personalized cancer therapy.

Chapter 5: Linking CIN and EVs in TNBC therapy

In chapter 5, we explore the relationship between CIN and EVs in TNBC and investigate the effects of MPS1 inhibitors on tumour metastasis. We find that CIN as induced by the MPS1 inhibitor reversine leads to an increased release of EVs that facilitate cell migration. Proteomic analysis of these CIN-induced EVs identifies EFEMP1, a key extracellular matrix protein, as a significant component driving the migration-promoting effect. Further exploration reveals that EFEMP1’s influence on cell migration is modulated by STAT1 signalling, underscoring a complex regulatory mechanism underpinning CIN-induced changes in the TME. In vivo validation in a zebrafish xenograft model confirms a pivotal role of EFEMP1-enriched EVs in promoting cancer cell migration. The findings underscore EFEMP1 as a critical link between CIN and metastatic potential in TNBC, presenting it as a potential therapeutic target to curb the pro-metastatic influence of CIN-positive cancer cells on the TME. Through a comprehensive investigation of the paracrine effects of CIN-induced EVs, this work contributes to a deeper understanding of TNBC progression and opens new avenues for targeted intervention in CIN-driven cancers.

Chapter 6: General summary and discussion

This chapter provides a summary and discussion of the major findings of the work, along with addressing future research directions and study implications.
References


Desai PP, Narra K, James JD, Jones HP, Tripathi AK & Vishwanatha JK (2022) Combination of Small Extracellular Vesicle-Derived Annexin A2 Protein and mRNA as a Potential Predictive Biomarker for Chemotherapy Responsiveness in Aggressive Triple-Negative Breast Cancer. *Cancers (Basel)* 15


Yang J & Zhang C (2020) Regulation of cancer-immunity cycle and tumor microenvironment by nanobiomaterials to enhance tumor immunotherapy. WIREs Nanomedicine and Nanobiotechnology 12: e1612


