Unravelling the complexities of chromosomal instability in cancer: exploring molecular pathways and potential therapeutic targets
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CHAPTER 6

General summary and discussion
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Summary

This thesis thoroughly examines mechanisms of chromosomal instability (CIN) and their impact on cancer progression.

In chapter 2, we explored the paradoxical relationship between CIN and the tumour microenvironment, highlighting the therapeutic opportunities arising from this interaction.

CIN, common in solid tumours and haematological malignancies, leads to increased chromosome mis-segregation, resulting in aneuploidy. In this review, we discussed the connection between CIN and the tumour microenvironment (TME), and whether and how this interaction contributes to cancer progression.

This chapter critically examines the contribution of CIN to tumour recurrence, metastasis, and therapy resistance, all factors that lead to a poor prognosis. We examined how CIN affects the TME, acknowledging the active role of the TME in cancer progression. The review emphasizes how CIN modifies the TME to promote metastatic spread and resistance to traditional treatments.

Additionally, we evaluated the therapeutic implications of the between CIN cancer cells and their TME. By understanding how CIN shapes the TME, new therapeutic targets can be identified, and innovative strategies for treating cancer can be developed. This involved investigating how targeting CIN can potentially modify the TME to improve treatment outcomes.

Through this review, we emphasize the importance of considering both CIN and the TME in developing future cancer therapies. In the next chapter, we transitioned from the detailed examination of CIN's influence on the TEM to cancers characterized by Maternal Embryonic Leucine Zipper Kinase (MELK) overexpression, a trait frequently linked to CIN+ lymphomas.

In Chapter 3, we investigated HDAC4 as a potential therapeutic target in cancers with Maternal Embryonic Leucine Zipper Kinase (MELK) overexpression, a phenomenon particularly observed in CIN+ lymphomas. We identified potential therapeutic targets in cancers with overexpressed MELK. Despite frequent overexpression in various cancers, the functional role of MELK in oncogenesis is not fully understood. MELK plays a critical
role in cancer-related processes, such as tumour growth, chemotherapy resistance, and tumour recurrence, making it a promising target for therapeutic intervention.

In an attempt to utilize MELK overexpression for treatment, we conducted a high-throughput screening of a Saccharomyces cerevisiae mutant library. This screening aimed to identify genes essential for cell survival in the context of heightened MELK activity, effectively identifying synthetic lethal partners with MELK overexpression. This approach allowed us to uncover genes that, when inhibited or lost, could selectively compromise the viability of cells overexpressing MELK, offering a targeted strategy for cancer treatment. Our study identified two genes of interest: LAG2 and HDA3. LAG2 inhibits the Skp, Cullin, F-box containing (SCF) ubiquitin-ligase complex, while HDA3 is part of the HDA1 histone deacetylase complex.

In our study, we found that inhibiting the human homolog of HDA3, Histone Deacetylase 4 (HDAC4), causes synthetic lethality in cells with high MELK expression. This indicates that targeting HDAC4 could be a potential treatment strategy for cancers with MELK overexpression. Our research identifies HDAC4 as a relevant player in the MELK pathway and a potential drug target for MELK-overexpressing tumours.

Finally, this chapter discussed the implications of targeting HDAC4 in MELK-overexpressing cancers and how this approach could lead to new treatment strategies in oncology. Expanding on the investigation of HDAC4 as a potential therapeutic target discussed in Chapter 3, Chapter 4 shifts focus to a more comprehensive examination of CIN+ cancers. This involves the utilization of a CRISPR screen to identify genes that enhance cancer cell resistance to elevated levels of chromosomal instability (CIN).

In Chapter 4, we performed a CRISPR screen to identify genes that enable cells to withstand high levels of CIN, a common trait in CIN+ cancers, to understand better how cancer cells develop resistance to CIN. This screening was particularly focused on identifying genes that enhance resistance to Spindle Assembly Checkpoint (SAC) inhibitors, as these inhibitors often exploit CIN to target cancer cells. Our work revealed a crucial role for CDC20, exposing it as a critical gene that cancer cells rely on to tolerate CIN and counteract the effects of SAC inhibition. These inhibitors disrupt chromosome segregation during cell division, leading to CIN and, ultimately, cell death. Clinical trials are evaluating the clinical potential of these drugs for solid cancers, but there is an urgent need for biomarkers to predict their effectiveness.
Our work showed that CIN+ cancer cells are more sensitive to SAC inhibition than their CIN- counterparts. Delving into the molecular underpinnings of this vulnerability, we centred our investigation on CDC20, a key gene identified in our CRISPR screen as a significant activator of the anaphase-promoting complex (APC/C). We found that sensitivity to SAC inhibitors is more closely associated with the expression levels of CDC20 than those of APC/C.

Through a series of experiments, we found that depleting CDC20 resulted in longer metaphase, fewer mitotic errors, and a reduced sensitivity to SAC inhibitors. Interestingly, SAC inhibitors typically shorten the overall duration of mitosis, including metaphase. However, in CDC20-depleted cells, the extended time before anaphase onset allowed the spontaneous repair of mitotic errors, enhancing the cells’ ability to rectify aberrations and reducing their vulnerability to SAC inhibition. Aneuploid cells with high CDC20 expression had shorter metaphases and multiple mitotic errors, making them more sensitive to SAC inhibition in the long term.

This chapter suggested that high CDC20 expression could serve as a predictive biomarker for the effectiveness of SAC inhibition therapy. Targeting CDC20, as discussed in further detail, could exploit this aneuploidy-induced therapeutic vulnerability, providing a new approach to cancer treatment. Building on the discovery of CDC20’s critical role in cancer cell resilience against CIN and SAC inhibition, the following chapter shifts our focus to the dynamics between CIN and extracellular vesicles (EVs) in TNBC, marking a transition from understanding cellular resistance to exploring the expansive effects of CIN on intercellular communication and the metastatic process within the challenging landscape of TNBC.

In chapter 5, we explored the complex interplay between CIN and EVs in the complex context of TNBC. Upon treatment of TNBC cells with the MPS1 inhibitor reversine, we observed a significant increase in EVs production and release. We found that reversine-induced CIN EVs promote the migratory and invasive properties of TNBC cell lines.

Mass spec analysis of the contents of these EVs revealed that CIN+ EVs were enriched with the EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) protein originating from CIN+ TNBC and follow up experiments identified this protein as a significant facilitator of cell migration and invasion in cells, highlighting its crucial involvement in metastasis progression.

Upon further investigation, we found that the EFEMP1 expression is dependent on STAT1 signalling pathway. Moreover, our experiments demonstrated that EVs derived from cells
with reduced EFEMP1 levels exhibit a significant decrease in their capacity to enhance migration and invasion. This diminished effect is mainly attributed to their reduced influence on cellular adhesion, as observed in TNBC cell lines such as BT549 and MDA-MB-231.

Next, we utilized zebrafish xenograft models to validate the increased migratory activity of cells subjected to EFEMP1-enriched EVs in vivo. This experiment confirmed the involvement of these vesicles in the spread of metastatic cells throughout the fish. Importantly, our findings are supported by DepMap and GEPIA datasets, confirming the link between EFEMP1 expression and aneuploidy, highlighting EFEMP1's potential as a therapeutic target to combat metastasis in advanced breast cancer stages.

This chapter uncovers a potential therapeutic value of EFEMP1 in interventions designed to reduce the metastatic capacity of cancer cells affected by CIN. Our study thus not only contributes to the current knowledge of CIN in TNBC but also lays the groundwork for the potential development of targeted therapies that may substantially enhance outcomes in TBNC.
Discussion

Building on the findings of this thesis, several important questions have emerged for future investigation:

(1) HDAC4 in MELK-Expressing Cancers: What are the molecular mechanisms by which HDAC4 influences cancers with MELK overexpression, and how can this knowledge inform the development of targeted therapies?

In Chapter 3, our research identified Histone Deacetylase 4 (HDAC4) as a critical therapeutic target in cancers with MELK overexpression, signalling a new approach to cancer treatment (Zhou et al., 2021). The finding that inhibiting HDAC4 triggers synthetic lethality in cells overexpressing MELK not only paves the way for new targeting strategies but also warrants further exploration of the molecular mechanisms involved in this interaction (Zhou et al., 2021).

Although we have established an important role of HDAC4 in influencing the progression of MELK-overexpressing cancers, further exploration is needed to understand the specific molecular pathways involved. Previous studies have revealed different pathways through which HDAC4 affects cancer progression. HDAC4 has been found to suppress p21, promoting cancer cell progression (Kang et al., 2014), and to facilitate cancer cell migration and invasion through the FAK/Paxillin pathway (Du et al., 2014). The role of HDAC4 has been studied in various cancers, including nasopharyngeal carcinoma and esophageal carcinoma (Cheng et al., 2021; Zeng et al., 2016), and its impact on drug sensitivity, such as its control over sensitivity to cisplatin in gastric cancer through the p53-p73/BIK pathway (Spaety et al., 2019).

Furthermore, HDAC4 plays a role in promoting gastric cancer growth and metastasis through the autophagic degradation of MEKK3 (Zang et al., 2022), contributing to lung cancer tumorigenesis by deacetylating glutaminase (Wang et al., 2022), and impacting drug resistance, such as its role in tamoxifen resistance in ER-positive breast cancer cells (Ahmad et al., 2015).

The overexpression of MELK in different cancers has been associated with important oncogenic functions, indicating its potential as a therapeutic target for conditions like gastric and cervical cancer. Conversely, the inhibition of MELK has shown promising results in targeting cancer stem cells (Ren et al., 2019) and impacting clinical outcomes in diseases such as ovarian cancer (Ikeda et al., 2020).
Our future experiments aim to uncover the underlying molecular mechanisms by which HDAC4 affects MELK-overexpressing cancers. This work is crucial for advancing our comprehension of the specific roles these proteins play in oncogenesis, guiding the development of targeted therapies that capitalize on these insights to enhance treatment efficacy in MELK-prevalent cancers. By analysing these pathways, we aim to use this knowledge to enhance the precision of cancer treatments, particularly in cancers characterized by MELK overexpression.

(2) CDC20 and Sensitivity to SAC Inhibitors: In what ways does CDC20 overexpression in aneuploid cells enhance their sensitivity to SAC inhibitors, and how can this be utilized to tailor treatment strategies of aneuploid cancers?

In Chapter 4, we identified CDC20 as a primary determinant of sensitivity to SAC inhibitors, while also recognizing APC/C components as significant targets identified by our screen (Zheng et al., 2023). After discovering that overexpressing CDC20 in aneuploid cells may be an adaptation to the aneuploid state, we found that it not only promotes CIN and aneuploidy but also increases the cells' susceptibility to further CIN induction, such as that caused by SAC inhibitors (SACi) (Zheng et al., 2023). This suggests that increased CDC20 expression could be used as a prognostic biomarker for the effectiveness of SACi and other CIN-inducing therapies in various aneuploid cancer types (Zheng et al., 2023).

The overexpression of CDC20 is associated with poor prognosis in cancers such as colorectal cancer and diffuse large B-cell lymphoma (Sun et al., 2020; Maes et al., 2019; Wu et al., 2013). Our work is indicating that this overexpression might contribute to abnormal mitotic progression. CDC20 overexpression might also contribute to oncogenesis by other mechanisms, e.g. it was found to promote cell proliferation and invasion while inhibiting apoptosis in osteosarcoma cells (Shang et al., 2018; Gao et al., 2018). Furthermore, CDC20's role in suppressing apoptosis by targeting Bim for ubiquitination and destruction underscores its contribution to the aggressive behavior and apoptosis resistance in cancer cells (Wan et al., 2014).

CDC20 plays a critical role in aneuploid cells by regulating apoptosis and cell proliferation, making it a potential target for cancer therapy (Wang et al., 2015). Aneuploid cells, which undergo chronic hypo-osmotic-like stress and rely on sphingolipid homeostasis, are specifically susceptible to SAC inhibitors (Tsai et al., 2019; Cohen-Sharir et al., 2021). Our study extends these insights, proposing that impairments in SAC or CDC20 functionality might intensify this susceptibility. Additionally, the dependence of aneuploid cells on the
spindle assembly checkpoint and specific proteins like KIF18A is directly linked to their sensitivity to SAC inhibition (Cohen-Sharir et al, 2021; Marquis et al, 2021), suggesting a profound interplay with CDC20's governance over this critical cell cycle checkpoint.

Targeting CDC20 overexpression in aneuploid cells could be a strategic approach for customizing treatment in aneuploid cancers (Zheng et al, 2023). Inhibiting CDC20 has shown potential in suppressing tumour growth in conditions such as metastatic castration-resistant prostate cancer and improving sensitivity to drugs like docetaxel (Li et al, 2016). Building on this premise, the innovative approach of disrupting the spindle assembly checkpoint (SAC) by interfering with the Mad2-CDC20 interaction offers a coherent extension to the development of novel SAC inhibitors, thereby linking CDC20's role in aneuploid cancer cells directly to broader SAC inhibition strategies (VanGenderen et al, 2020; Wang et al, 2013).

Further studies and clinical trials are required to establish elevated CDC20 expression as a reliable biomarker in clinical settings. These studies should aim to correlate CDC20 expression levels in cancer patients with their response to SAC inhibitors and related therapies, validating the biomarker's predictive accuracy for therapeutic response.

(3) CIN, EVs, and TNBC: How do CIN-influenced EVs, particularly those enriched with EFEMP1, drive the metastatic process in triple-negative breast cancer, and can they be effectively targeted in treatment strategies?

In Chapter 5, we explore the connection between CIN, EVs enriched with EFEMP1, and their role in driving the metastatic process in TNBC. We focus on how EFEMP1-enriched EVs, facilitated by STAT1 signalling, increase cell migration and invasion in TNBC, a highly metastatic and poor-prognosis cancer type.

In our study, we report the application of reversine to significantly enhance the production and release of EVs in TNBC cell lines BT549 and MDA-MB-231, showing a novel aspect of reversine's impact on cancer cell biology. Via proteomic analysis, we found that these EVs are highly enriched with EFEMP1, a molecule has been reported be able to suppress and promote tumour growth (Baroni et al, 2016; Cosentino et al, 2020; Livingstone et al, 2020). Specifically, EFEMP1 has been shown to activate the AKT signaling pathway, facilitating ovarian cancer invasion and metastasis (Yin et al, 2016). Conversely, it acts as a tumour suppressor in glioma, altering the extracellular microenvironment and modulating critical oncogenic pathways (Hu et al, 2011). Furthermore, EFEMP1 intensifies tumour progression in fibroblasts through the action of miR-9, which suppresses EFEMP1
expression, thereby transforming normal fibroblasts into cancer-associated fibroblasts (CAFs) and increasing chemotherapy resistance in adjacent TNBC cells (Cosentino et al, 2020). This complexity underlines the significance of our findings that CIN-induced EVs, enriched with EFEMP1, could modulate the tumour microenvironment in divergent ways.

The potential of EFEMP1-enriched EVs as reliable biomarkers for TNBC progression or treatment response is an area worth exploring. One possible direction is to quantify EFEMP1 levels in clinical samples, including assessing EFEMP1 enrichment in tumour tissues and blood samples of TNBC patients, to determine the correlation between EFEMP1 levels and TNBC progression.

As EFEMP1-enriched EVs with are associated with increased metastatic capacity from our findings, it is worthwhile to explore the therapeutic implications of EFEMP1 in EVs. In other words: could targeting EFEMP1 or its regulatory pathways offer new approaches for TNBC treatment?

Our research elucidated how CIN-induced upregulation of EFEMP1 can influence the TEM and drive metastatic behaviour; we highlight the potential of targeting EFEMP1-mediated pathways to disrupt these pro-metastatic processes in TNBC treatment strategies.

Recent studies discuss how structural chromosome instability in breast cancer may lead to packaging cytoplasmic DNA into EVs (Siri et al, 2021). Further research is warranted to unravel the effects of CIN on the composition of EVs. Specifically, the focus should be on the other important biomolecules within these vesicles. Future studies should employ comprehensive RNA and DNA sequencing analyses of cells experiencing CIN, aiming to explore a broad spectrum of mechanistic pathways that potentially shape the EV profile in the context of CIN, especially the accumulation of EFEMP1.

In conclusion, understanding the interaction between CIN, EFEMP1, and EVs in TNBC is crucial for developing therapies targeting aneuploid cancers. The role of STAT1 in mediating the effects of EFEMP1-enriched EVs from CIN cells, as well as the broader implications of EVs in TNBC and other cancer types, remains an important area for future exploration. This involves further studying the effects of reversine on CIN and the resulting EV production, potential off-target effects of MPS1 inhibitor, and the translation of findings from in vitro to in vivo environments. Addressing these questions through additional animal studies and patient material will advance our understanding and potentially yield improved treatment strategies for TNBC.
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