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Exploring the consequences of chromosomal instability in cancer

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Exploring the consequences of chromosomal instability in cancer

Lin Zhou

The work described in this thesis was conducted at the European Research Institute for the Biology of Ageing (ERIBA) in the Laboratory of Genomic Instability in Development and Disease, University of Groningen, Groningen, the Netherlands.

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Exploring the consequences of chromosomal instability in cancer

PhD thesis

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on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

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Wednesday 12 January 2022 at 12.45 hours

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GENERAL INTRODUCTION AND THESIS OUTLINE

Chromosomal instability (CIN) is a process leading to chromosomal segregation errors. Aneuploidy, an abnormal number of chromosomes, is the consequence of chromosomal instability. CIN is a hallmark of human cancer that is associated with tumour heterogeneity, metastasis, and therapeutic resistance, which indicates that CIN is a promising target for cancer therapy. However, the role of CIN in tumour development and the molecular mechanisms that underlie CIN are still unclear. The main aim of this thesis is to investigate the multiple consequences of CIN and aneuploidy, and to use this knowledge to explore strategies that can be exploited to selectively target tumours displaying CIN.

In **Chapter 1**, we summarize the key consequences of CIN and aneuploidization, and we discuss strategies following these findings that can be exploited in cancer therapy. CIN is initially detrimental to cells and triggers multiple cellular stress responses that include cell cycle arrest, cell death, and immune cell activation. However, cancer cells can develop aneuploidy-tolerating mechanisms to cope with CIN to overcome these aneuploidy-imposed stresses. Thus, targeting such aneuploidy-coping mechanisms is considered a promising therapeutic approach. We also discuss specific strategies to target tumours displaying a CIN phenotype, including CIN exacerbation, targeting of cellular stresses, (re)activation of the immune system, and induction of senescence.

In **Chapter 2**, we review the consequences of aneuploidy, but now focusing on different model systems used and the aneuploidy-tolerating pathways discovered in these. We discuss how small and large screen efforts have been and can be used to better understand the consequences of CIN and resultant aneuploidy including the main advantages and disadvantages of the various screening systems. We also discuss how we can exploit the outcomes from such screens to improve future cancer therapy.

To understand the behaviour of aneuploidy and CIN in human primary tumours, we investigate basal cell carcinoma (BCC) in **Chapter 3** as a model for cancer for which tumours are readily available due to the commonness of BCC. Single-cell whole-genome sequencing for various BCCs allows us to classify BCCs into three types according to their karyotypes: near-euploid, stably aneuploid, and CIN. We describe how BCCs can be stratified based on karyotype landscape and that this can be linked to specific molecular features. While alterations in the Hedgehog pathway are common across all BCCs, we find, in addition, that aneuploid BCCs exhibit a metabolic signature, and that CIN BCCs display an increase in genes involved in DNA repair, suggesting that

ongoing CIN *in vivo* leads to DNA damage. Altogether, these data suggest that different CIN rates in cancer cells could well affect their response to treatment. Moreover, our studies could further help to target advanced or metastatic BCC cancers.

In **Chapter 4**, we describe how CIN leads to senescence, a process that is generally more associated with DNA damage or replicative ageing of non-immortalized cells. Senescence is regarded as an essential mechanism to prevent tumorigenesis. Thus the modulation of the drivers of senescence might help to target CIN tumours. To investigate the mechanisms underlying CIN-induced induction of senescence, we performed a CRISPR screen to find genes that help cells bypass senescence. We describe how the inactivation of the cell cycle genes Cdc20 and Cdc27, and transcriptional adapter proteins Tada1 and Tada2b might help bypass CIN-induced senescence. Furthermore, we validate that loss of Cdc20 increases the survival of cells in which we induce CIN, which suggests that inactivation of Cdc20 could help cells cope with spindle assembly checkpoint inhibitors such as reversine.

To identify specific vulnerabilities of tumours with a CIN phenotype, in **Chapter 5**, we investigate the role of the serine/threonine-protein kinase MELK, which is frequently overexpressed in aneuploid tumours. We find that loss of MELK is well-tolerated by cells and that overexpression of MELK does not trigger CIN phenotypes by itself and therefore fails to explain why MELK is specifically overexpressed in aneuploid cancers. To selectively target cells that overexpress MELK, we therefore search for synthetic lethal interactions with MELK overexpression. To this aim, we set up a genetic screen in *Saccharomyces cerevisiae* to identify mutations that are lethal to cells that overexpress human MELK. Our screen reveals a synthetic lethal interaction between inhibition of HDA3 and MELK overexpression in yeast, which we confirm in human cells when we inhibit the HDA3-human homolog HDAC4 in combination with overexpression of MELK. This work might provide a new angle of how to exploit MELK overexpression in cancer therapy.

Finally, **Chapter 6** summarizes and discusses the major findings and the potential use of our findings for the future.