Chapter 1

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Throughout the human genome there are many sites where replication can be initiated, so-called ‘replication origins’. During G1-phase the replication machinery assembles onto the DNA thereby “licensing” the origin. Upon origin ‘firing’ the bidirectional replication fork is formed and DNA replication starts. The process of DNA duplication is continuously challenged, leading to slowing and stalling of replication forks. Stalled replication forks need to be stabilized and protected from degradation to prevent the accumulation of DNA lesions. Along with many other proteins, BRCA1 and BRCA2 are key components that protect stalled replication forks from nucleolytic degradation of nascent DNA. Thus, mutations in BRCA1 or BRCA2 impair the process of fork protection and result in fork degradation or fork collapse. The replication fork protection functions of BRCA1 and BRCA2 become increasingly important during conditions of replication stress, a state of global replication fork slowing and stalling.

An important cause of replication stress in tumors is the amplification of proto-oncogenes, for example CCNE1 (encoding Cyclin E1). The overexpression of Cyclin E1 results in the unscheduled firing of replication origins, which causes collisions between the translation and replication machineries. Additionally, Cyclin E1 overexpression exhausts the nucleotide pool, which also results in replication stress. The amplification of CCNE1 is frequently observed in genomically unstable tumors, including high-grade serous ovarian cancer (HG-SOC) and triple negative breast cancer (TNBC). Moreover, CCNE1 amplification in TNBC and HG-SOC characterizes the patient subgroups with the poorest prognosis.

Division of genomic content occurs during mitosis. To prevent loss or gain of genomic content, it is crucial that DNA lesions that arise during interphase are not transferred into mitosis and transmitted into the newly formed daughter cells. To prevent accumulations of DNA lesions, cells rely on cell cycle checkpoints, especially the G2/M checkpoint.

Cell cycle checkpoint do not function as linear pathways, but rather as parallel pathways, each responding to different DNA lesions. DNA double-stranded breaks (DSBs) trigger the activation of the cell cycle checkpoints via activation of the MRN complex and the ATM kinase. Activated ATM phosphorylates many downstream targets, including the p53 transcription factor. p53 prevents S-phase entry, thereby installing a G1/S checkpoint. In parallel, ATM activates the CHK2 kinase, which results in inactivation of the...
DNA lesions in mitosis

DNA lesions or erroneous physical connections between chromosomes can prevent correct chromosome segregation over the two daughter cells. To ensure the faithful segregation of the two copies of each chromosome, cancer cells require mechanisms that cope with DNA lesions during mitosis. Although during mitosis, most canonical DNA damage repair pathways are inactivated, some DNA repair pathways remain available. MUS81-EME1 and SLX1-SLX4 nucleases are still able to sever connected DNA molecules during mitosis. Furthermore, DNA molecules that are still attached at onset of anaphase can form ultrafine DNA bridges (UFBs). These UFBs are resolved by BLM-Toposiomerase-RMI1/2 and Rif1, which facilitate UFB breakage to allow chromosome segregation. Although the DNA repair pathways that are available during mitosis allow chromosome segregation, they result in loss of genomic content.

Recently, it has been described that the mitotic DNA synthesis (MiDAS) repair pathway aids in the process of faithful chromosome segregation by synthesizing DNA at under-replicated regions, in the early stages of mitosis. POLD3 has been described to be the main polymerase involved in MiDAS. Furthermore, several additional proteins have been described to be involved in MiDAS, including SLX4, the MUS81-EME1 endonuclease complex, RAD52, FANCD2 and RECQL5. Despite the seemingly important role of MiDAS, the underlying molecular mechanism remains unclear. Specifically, it is unknown how replication forks can be initiated during mitosis and whether MiDAS reflects events in mitosis or late G2 phase.

Taken together, cancer cells experience an increased load of DNA lesions as a result of high amounts of replication stress or mutations in DNA repair genes. These lesions are not always repaired before mitotic onset, which potentially fuels genomic instability and may negatively impact on cellular viability. Cancer cells must have evolved mechanisms to cope with high levels of replication stress, mitotic DNA lesions and subsequent genomic instability. Targeting these pathways that mitigate the increase in replication stress or mitotic DNA lesions in cancer cells might be a strategy to selectively kill these cancer cells. To be able to do this, a better understanding is needed of the molecular mechanisms dealing with DNA lesions in mitosis.
AIM OF THIS THESIS

The overall aim of this thesis is to dissect the mechanisms of how replication-born DNA lesions affect mitotic behavior of cancer cells to improve treatment.

Outline of this thesis
In chapter 2 of this thesis, the phenotype of “BRCAness” is discussed and how BRCAness tumors have impaired DSB break repair, compromised replication fork protection and increased sensitivity to replication-blocking agents. We describe in detail the functions of HR genes in DSB repair, and in replication fork protection. Moreover, we discuss how BRCAness tumors increasingly depend on alternative DNA repair pathways. These alternative DNA repair pathways are often error prone and leave specific genomic scars. Due to these specific scars in BRCAness tumors, next-generation sequencing approaches can be used to identify BRCAness tumors, beyond the mutational status of the prototypical HR genes. Identification of BRCAness tumors based on genomic scars without a BRCA1 or BRCA2 mutation may be clinically relevant as these tumors may respond favorably to specific treatment.

BRCA1/2 deficient tumors have an increased sensitivity towards PARP inhibitor treatment. However, while PARP inhibitors showed great promise initially, tumors typically develop resistance and relapse. In chapter 3, we investigate how the synthetic lethal interaction between PARP inhibitor and BRCA2 deficiency can be potentiated. To this end, we aim to prematurely enforce mitotic entry in BRCA2-deficient cells which are treated with PARP inhibitor. Specifically, mitotic entry was achieved through inhibition of the G2/M checkpoint kinase ATR. We determined the timing of mitotic entry by flow cytometry and assessed genomic instability by microscopy and single-cell DNA sequencing.

Untimely firing of replication origins as a result of overexpression of the proto-oncogene Cyclin E1 is a well described cause of replication stress. Replication stress fuels genomic instability and fuels tumor progression. To survive high levels of replication stress, tumors depend on pathways to deal with these DNA lesions. We hypothesized that increased replication-associated DNA lesions as a consequence of Cyclin E1 overexpression can therapeutically be exploited. To this end, in chapter 4 we developed inducible Cyclin E1 overexpression models in the untransformed RPE-1 cells. By using DNA fiber analysis, we investigated if amplification of CCNE1 resulted in replication stress and whether subsequent DNA lesions were transferred into mitosis. We monitored mitotic behavior upon oncogene induced replication stress by using immunofluorescent microscopy and live-cell microscopy. Additionally, we attempted to specifically target cells with oncogene induced replication stress by inhibition of the G2/M cell cycle checkpoint kinases ATR and WEE1.

In chapter 5 we further explored the consequences of Cyclin E1 overexpression. Specifically, we investigated if cells need to progress through S phase with high Cyclin E1 levels to enter mitosis with DNA lesions. We assessed MiDAS in Cyclin E1-overexpressing cells using microscopy and analyzed whether Cyclin E1 cells depended on MiDAS to cope with replication stress.

The endonuclease MUS81 has been described to be involved in the process of MiDAS. In chapter 6 we aimed to isolate mitotic DNA foci by performing immune-precipitations of MUS81. In order to do so, we engineered cells with GFP-tagged MUS81, MUS81 is known to localize to MiDAS foci. We induced replication stress in these cells and immuno-purified the GFP-tagged proteins both in mitotic and interphase fraction of the cell populations. We then analyzed the interactome of MUS81 using mass-spectrometry.

Finally, in chapter 7 the results and conclusions obtained in the different chapters are summarized and discussed.
REFERENCES


