CHAPTER 1
INTRODUCTION & OUTLINE
OF THE THESIS
INTRODUCTION

*Maintenance of genome integrity by DNA damage signalling and repair*

Essential for life, is that every cell delivers its genetic material, unchanged and intact, to its offspring. However, each cell in the human body receives tens of thousands of DNA lesions per day. These lesions can either be caused by endogenous factors, such as DNA mismatches introduced during DNA replication, or environmental agents of which ultraviolet light (UV) is the most prevalent. To solve these lesions, cells are equipped with several systems – together called the DNA damage response (DDR) – to detect DNA damage, signal its existence and facilitate its repair. As there are many different types of lesions which require a different type of repair, a wide diversity of DNA repair mechanisms exists. For example, bulky DNA lesions are repaired by nucleotide excision repair (NER), while DNA double-strand breaks are repaired by non-homologous end-joining (NHEJ) or – when the sister chromatid is present – by homologous recombination (HR). Although most systems comprise nuclease, polymerase and ligase enzymes, the specific proteins are largely distinct.

Ultimately, DNA lesions can block transcription and DNA replication, and if they are not repaired, mutations or larger genomic aberrations can arise. Therefore, cells deficient for DDR or DNA repair factors generally display increased sensitivity towards DNA-damaging agents and often cause diverse human diseases, such as cancer and neurodegenerative disorders. For instance, women with a deleterious germline mutation in HR-genes BRCA1 or BRCA2 have up to 70% risk of developing breast cancer by the age of 70.

*Time for repair is generated by cell cycle arrest*

To maintain genome integrity, passage of DNA lesions to daughter cells should be prevented. Therefore, there is a close connection between the DDR and cell cycle regulation. Depending on the cell cycle phase, different checkpoint pathways are activated upon DNA lesions. These checkpoints induce a cell cycle arrest, after which cells resume cell cycle progression once damage has been repaired, or undergo permanent cell cycle arrest or apoptosis in case of unrepairable DNA lesions. These checkpoints operate by regulating the main drivers of the cell cycle; the Cyclin/Cyclin-dependent kinase (CDK) complexes. To promote cell cycle progression, these Cyclin/CDK complexes require post-translational modifications to be activated. In response to cellular insults, kinase-driven signalling networks control the inhibitory phosphorylation of CDKs. In case of the G2/M cell cycle checkpoint, CDK1 needs continuous phosphorylation at tyrosine 15 (Y15) to prevent premature initiation of mitosis. In normal situations, CDK1 is phosphorylated at Y15 by the kinase WEE1 and dephosphorylated by one of the CDC25 phosphatases. In situations of DNA damage, the downstream DDR kinases CHK1 and CHK2 inhibit CDC25 phosphatases through direct phosphorylation, which leads to a block in CDK1 activation (Fig. 1).
Targeted anti-cancer therapies

Currently, radiotherapy and chemotherapy are the mainstays of treatment for most tumors. Both radio- and chemotherapy kill rapidly growing cancer cells by inducing high levels of DNA damage. Ultimately, these extensive amounts of DNA lesions will trigger apoptosis or lead to mitotic catastrophe. However, not all tumors, even within one tumor class, are equally sensitive to DNA damaging agents. For example, chemotherapeutics were observed to be less effective when tumors comprise enhanced DNA repair capacity or alterations in the apoptotic pathway\textsuperscript{11,12}. To more effectively eliminate tumors, classical chemotherapy and radiotherapy can be combined with inhibitors or antibodies targeting specific pathways for tumor survival. This so-called ‘targeted therapy’ is based on three rationales. The most common one is referred to as ‘oncogene addiction’. This rationale refers to the notion that cancer cells often become dependent on the activity of oncogenes for their growth advantage\textsuperscript{13,14}. Targeting such an oncogene can cause effective and specific tumor cell killing. For instance, tamoxifen and trastuzumab treatment in estrogen receptor-positive and HER2-overexpressing breast cancers, respectively, are frequently used manners to target oncogene addiction\textsuperscript{15,16}. The second concept of targeted therapy is ‘synthetic lethality’. This refers to a situation where a defect in one gene, ‘gene A’, causes dependency on a second gene\textsuperscript{17,18}. Targeting this second gene, ‘gene B’, will only be lethal in tumors with a defect in gene A, which is called synthetic lethality. Thus, in tumors without a defect in gene A, targeting gene B will have minimal effects on cell survival. The prototypical example of a synthetic lethal combination is the treatment of BRCA1/2 defective tumors with Poly-(ADP-Ribose)-polymerase (PARP) inhibitors\textsuperscript{19}. The third rationale of targeted therapy is ‘non-

\begin{figure}
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\includegraphics[width=\textwidth]{DNA_damage_response_cell_cycle_regulation.png}
\caption{DNA damage response and cell cycle regulation. DNA double-strand breaks and junctions that arise at stalled replication forks activate ATM and ATR, respectively. ATM phosphorylates – and hence – activates CHK2, which in turn activates p53 that arrests the cell cycle at the G1/S phase. In addition, CHK2 as well as CHK1 (which is phosphorylated by ATR) and MK2 (which is phosphorylated by p38) initiate the S/G2 or G2/M checkpoint arrest by inhibiting the CDC25 phosphatases. Alternatively, CHK1 activates WEE1, which phosphorylates and thereby inhibits CDKs to prevent S/G2 or G2/M transition. Phosphorylation (red ‘p’) and dephosphorylation (grey ‘p’) events are indicated.}
\end{figure}
oncogene addiction”, which refers to tumors becoming dependent on genes which are not depicted as oncogenes. Genes involved in controlling the DDR and cell cycle regulation, including CHK1, ATR, WEE1 and PLK1, are good examples of non-oncogenes, on which tumor cells can become dependent for their survival. Modulation of cell cycle progression in the presence of DNA damage is seen as a potentially effective combination strategy in anti-cancer therapy. Cells are equipped with several cell cycle checkpoints that can arrest passage through the cell cycle until defects are repaired. Therapeutic inhibition of cell cycle checkpoints can deregulate cell cycle control and improperly force cell cycle progression, even in the presence of endogenous or chemotherapy-induced DNA damage. One example to chemically deregulate cell cycle control is inhibition of the cell cycle checkpoint kinase WEE1. Chemical inhibitors of WEE1 have recently been tested in phase-II clinical trials, either as a single agent or in combination with chemotherapy, and show promising results.

Two substantial challenges of targeted anti-cancer therapies are 1) that their successful use in the clinic requires patient selection prior to treatment, and 2) the observation that tumors frequently develop resistance to molecularly targeted anti-cancer agents. The need for proper patient selection follows the rationale underlying molecularly targeted anti-cancer agents; targeted agents are aimed at specific vulnerabilities of cancer cells. Such vulnerabilities are not present in each tumor, even within tumors of the same tumor type. Hence, not all tumors will be sensitive to a particular treatment. Moreover, sub-optimal treatment (i.e. of tumors that are not very sensitive to a targeted agent) can cause the emergence of resistant tumor cells that show increasingly aggressive behavior and are more difficult to treat. These notions also hold true for chemotherapeutic agents that cause DNA damage, and targeted agents that interfere with the DNA damage response. Because of the multitude and complexity of ways that tumors can cope with DNA damage, it is extremely challenging to select patients and predict sensitivity to genotoxic treatment regimens. For instance, tumor cells may be able or unable to repair the inflicted damage, do or do not engage cell cycle arrest, and may be more or less prone to induce programmed cell death.

To ultimately achieve the development of successful molecularly-targeted anti-cancer agents and implement efficient patients selection, we need to better understand at the cell biological level how tumor cells cope with DNA damage and which (epi)genetic factors determine these cellular cell fate decisions. The notion that signal transduction pathways are not static factors, but rather change during the development of cancers or in response to treatment further challenges this goal.

**AIM**

The overall aim of the research described in this thesis is to uncover factors that determine cell fate after DNA damage. This aim will be addressed in the context of intrinsic DNA damage, inflicted by defective DNA repair, or extrinsically-induced DNA damage through cell cycle checkpoint inhibition or cisplatin administration. To answer these questions, two different types of screens are employed:

I)  Loss-of-function haploid genetic screens
II) A systems biology-based screen on checkpoint activity and chemo sensitivity
OUTLINE OF THE THESIS

Deregulation of cell cycle control by therapeutic inhibition of cell cycle checkpoints can force cells to progress through the cell cycle, even in the presence of DNA damage. For example, WEE1 inhibition results in premature mitotic entry in the presence of unrepaired DNA damage. Mitotic cells respond differently to DNA damage when compared to interphase cells, but how exactly cells respond to DNA damage during mitosis and which fate they have remains largely unclear. In Chapter 2, we therefore reviewed the scientific literature, to discuss the molecular details concerning DDR signaling during mitosis as well as the cellular fate after encountering DNA breaks during mitosis.

Recent findings have indicated that various mitotic kinases, including CDK1, inactivate DNA damage checkpoint proteins when cells enter mitosis and thereby inhibit DNA repair. To test if targeted modulation of CDK1 activity could be used to affect DNA repair in interphase cells, we studied the effects of forced CDK1 activation in Chapter 3. Specifically, we tested if the clinically-used WEE1 inhibitor MK-1775 could enforce CDK1 activation and subsequently alter HR repair. After assessing the cytotoxicity and radiosensitizing capacity of WEE1 inhibition in non-transformed and cancer cells, we analyzed the effects of WEE1 inhibition on the DNA damage response. Finally, we assessed the effects of WEE1 inhibition on HR repair using \textit{in vivo} endonuclease-induced HR-assays, and studied the responsible CDK1 substrates involved in DNA repair.

Inhibition of WEE1 is considered an attractive anti-cancer therapy for \textit{TP53}-mutant tumors. However, additional factors besides p53 inactivation may determine WEE1 inhibitor sensitivity. To optimally facilitate patient selection for WEE1 inhibition and undercover potential resistance mechanisms, identification of these currently unknown genes is necessary. Therefore, the aim of Chapter 4 was to identify gene mutations that determine WEE1 inhibitor sensitivity. Using an unbiased functional genetic screen we searched for gene mutations that confer resistance to WEE1 inhibition in a \textit{TP53}-mutant background. We subsequently validated whether depletion of these genes could rescue the cytotoxic effect of WEE1 inhibition in other cancer cell lines as well. Furthermore, we studied the molecular link between the identified genes and WEE1 inhibitor resistance by examining DNA damage accumulation and cell cycle progression using flow cytometry and live-cell imaging.

Increasingly, we realize that the DDR is not functioning as one linear signaling pathway. Rather, it employs several parallel pathways that display extensive crosstalk and feedback control. As a result of this complexity, it proves very difficult to a \textit{priori} predict which tumors will be resistant to DNA damaging agents and which therapeutic interventions could be used to sensitize tumors to DNA damaging agents. Therefore, a detailed understanding of how molecular signals are integrated and influence chemosensitivity is necessary to optimize the prediction of treatment efficacy and allow rational choices of effective combination therapies. In Chapter 5, we investigated which signaling pathways drive chemosensitivity in triple negative breast cancer (TNBC), an incompletely understood tumor type with a worse overall prognosis when compared to other breast cancers. To investigate in a systematic manner which molecular signals drive checkpoint leakiness and chemosensitivity in TNBC, we used mathematical
modeling of quantitative time-resolved cell signaling analyses with phenotypic response data of two sensitive and insensitive TNBC cell lines, treated with varying doses of cisplatin. Genomic instability, a process in which (tumor) cells progressively lose or gain chromosomal fragments, characterizes many aggressive tumors and is often caused by defective DNA repair. BRCA2 is one of the genes involved in double strand break repair and replication fork stability, which is observed to be mutated in genomically instable tumors. Surprisingly however, whereas loss of BRCA2 is tolerated in tumor cells, it is deleterious for survival of normal cells. In Chapter 6, we performed a loss-of-function haploid genetic screen to unravel how tumor cells deal with impaired genome maintenance induced by BRCA2 inactivation. After assessing which gene mutations confer resistance to BRCA2 loss in a TP53-mutant background, we validated whether depletion of these genes also rescued BRCA2 loss and related factors of replication stress resolution in various human and murine cancer models. In addition, we studied the molecular mechanisms underlying the identified gene mutations in BRCA2 deficient models with ELISA, flow cytometry and mass spectrometry. Finally, Chapter 7 summarizes and discusses the experimental results obtained in the previous chapters.

REFERENCES


