General introduction and thesis outline
**General introduction**

Cervical cancer is a common malignant disease among women worldwide and contributes to an estimated 8% of all cancer-related deaths [1]. In patients with locally advanced cervical cancer (LACC) radiotherapy plus since 1999 also platinum-based chemotherapy is standard of care. The addition of chemotherapy improved 5-year survival with around 6% [2]. However, there is still room for major improvement with 5-year survival rates in the advanced stages of cervical cancer, varying from 66% in stage IIB to 22% in stage IVA [3]. Current conventional treatment modalities seem to have reached their limits.

One of the factors thought to be involved in treatment failure is the ability of tumor cells to repair chemoradiation-induced DNA damage. Chemotherapy and radiotherapy exert their anticancer effects by generating high levels of DNA damage to promote apoptosis. As a consequence the DNA damage response (DDR) is activated in tumor cells to cope with therapy-induced DNA lesions [4]. A proficient DDR enables tumor cells to repair therapy-induced DNA-damage in contrast to tumor cells in which DDR components are inactivated [5-10]. Therefore, inhibition of the DDR as novel therapy to sensitize tumor cells for chemoradiation is currently investigated in preclinical and clinical studies.

Cervical cancer arises almost invariably from persistent infection with high-risk human papilloma viruses (HPV) type 16 or 18, which leads to expression of viral oncoproteins, including E6 and E7 [11]. E6 and E7 promote cervical carcinogenesis by binding to tumor suppressor proteins, respectively p53 and the retinoblastoma protein 1 (Rb1) [12,13]. This results in enhanced cellular proliferation, blocked apoptosis and defective cell cycle control at the G1/S checkpoint. In response to aberrant proliferation, the DDR is activated [14,15]. In normal cells this results in sustained p53 activation to promote apoptosis and cell cycle arrest [4]. A critical step in this response is the activation of the G1/S checkpoint by p53 [16]. The HPV-mediated oncogenesis of cervical cancer results in p53-defective cells that rely on residual checkpoint capacity for survival after DNA damage.

Normal cells are continuously exposed to different exogenous and endogenous agents resulting in a variety of DNA lesions. In order to counter these lesions and thereby maintain genomic integrity, normal cells respond to these DNA lesions via the DDR thereby directing cell cycle arrest, DNA repair and programmed cell death. Therefore, DDR inhibition is considered a potential treatment strategy to confer chemoradiation in cancers. Furthermore, the DDR has been proposed as an anticancer mechanism that prevents tumorigenesis [14,15]. Not surprisingly, in most cancers, somatic mutations in DDR components, like TP53, are observed [17]. However, the DDR consists of multiple, partially overlapping, pathways. This results in re-wired DDR signaling in cancer cells, in which specific DDR pathways partly take over defects in compromised DDR pathways. As a consequence, these tumor cells are more dependent on specific DDR pathways compared to normal cells. Therefore, specific inhibition of these DDR axes is considered to be preferentially cytotoxic for tumor cells over normal cells. This concept is called ‘synthetic lethality’. In p53-defective cancers, like cervical cancer, the G1/S cell cycle checkpoint is abrogated making these cancers more dependent on remaining cell cycle checkpoints. Subsequently, inhibition of these residual checkpoint pathways selectively exacerbates toxicity for chemo- or radiotherapy in p53-defective cancer cells [18]. Taken together, DDR defects in tumor cell are suggested to be an attractive ‘Achilles heel’ to exploit therapeutically. Cervical cancer might also benefit from this strategy.
The aim of this thesis is to improve chemo- and/or radiosensitization by DDR dysregulation in preclinical in vitro and in vivo cervical cancer models.

**Thesis outline**

In Chapter 2, we reviewed literature retrieved from PubMed (including Medline), Embase and the Cochrane database about the use of DDR inhibitors in the context of potentiating the response to chemoradiotherapy in cervical cancer and summarized the current knowledge from preclinical and clinical studies, including current clinical trials (as registered on www.clinicaltrials.gov). Both cisplatin and ionizing radiation introduce high levels of DNA double-strand breaks (DSBs). DSBs generation is considered as extremely toxic for tumor cells. However, the cytotoxic effects of DSBs are countered by the activity of DDR kinases, which control repair of these DSBs. A central regulator in DSB signaling is the ataxia telangiectasia mutated (ATM) kinase, which is autophosphorylated upon DSBs. Therefore, ATM inhibition was suggested as a strategy to improve radiosensitization in tumor cells. To investigate this hypothesis, we investigated in Chapter 3 the predictive value of ATM and one of its substrates, p53-binding protein-1 (53BP1) and their potential as therapeutic targets in cervical cancer. To this end, we analyzed the effects of stable depletion of ATM or 53BP1 in cervical cancer cell lines on cell cycle arrest and radiosensitivity. Finally, to explore the predictive value of ATM and 53BP1 in LACC patients, we performed immunohistochemistry for (non-)phosphorylated ATM and 53BP1 in 375 tumor samples of chemoradiotherapy-naïve cervical cancer patients.

The DDR consists of multiple kinases. In Chapter 4 we analyzed the presence and activation status of several key DDR kinases in cervical cancer. Due to HPV-mediated p53 inactivation, cervical cancers are thought to rely on other DDR pathways for their survival. As a consequence, the DDR kinases in these pathways are interesting therapeutic targets to increase sensitivity to cisplatin and ionizing radiation. However, which DDR kinase is most efficient as therapeutic target in cervical cancer is not clear. We therefore focused in this chapter on ATR (ataxia telangiectasia and RAD3-related protein), DNA-PK (DNA-dependent protein kinase), Chk1 (checkpoint kinase 1), Chk2 (checkpoint kinase 2) and MK2 (MAPKAPK-2). We evaluated their phosphorylated and non-phosphorylated expression levels in tumor tissues of cervical cancer patients. Subsequently, using specific chemical inhibitors for ATM (KU-55933), ATR (NU6027), DNA-PK (KU-0060648), Chk1/2 (AZD7762) and MK2 (MK2 inhibitor III), we investigated the effects of their inactivation on sensitization for cisplatin and ionizing radiation. Finally, we assessed sensitizing effects in combined treatment with cisplatin and radiation, which resembles current therapy strategy in clinic.

Cervical cancers rely mainly on the G2/M cell cycle checkpoint for repairing DNA breaks. A key controller to halt cells in G2 is the Wee1 kinase. This kinase is abundantly expressed in various cancer types and preclinical studies reported tumor growth inhibition with Wee1 inhibitor monotherapy and a synergistic anti-tumor effect when combined with chemotherapeutic agents or ionizing radiation. Therefore, we hypothesized that Wee1 inhibition might result in beneficial effects in cervical cancer. In Chapter 5 we immunohistochemically evaluated Wee1, expression levels in tumor samples of 204 cervical cancer patients. Subsequently, we investigated the efficacy of
Wee1 inhibition alone or as sensitizer for cisplatin in different human cervical cancer cell lines. In addition, we studied the underlying molecular mechanisms that contribute to the observed differential effects of Wee1 inhibition. Specifically, we investigated how HPV copy numbers in our panel of cervical cancer cell lines are related to differential efficacy upon Wee1 inhibition.

Clinical translation of candidate drugs found in preclinical experiments, is limited by the large number of drug candidates as well as unsuitable pharmacodynamics properties for \textit{in vivo} testing [19]. Also these novel therapeutic modalities should be clinically evaluated in comparison with current standard chemoradiotherapy. Most preclinical studies, however, only investigate novel therapeutics as monotherapy or in combination with either a genotoxic agent or ionizing radiation, but not in a combined setting [19]. In Chapter 6 we aimed to establish the chorioallantoic membrane (CAM) assay as a platform to evaluate chemo-radiotherapy sensitizing compounds using a 3-dimensional \textit{in ovo} cervical cancer model.

Conclusively, in Chapter 7, we summarized the experimental results in this thesis, discussed their implications and addressed remaining future challenges.
References


