Review

Therapeutic opportunities in cervical cancer: targeting the DNA damage response.

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Abstract

Cervical cancer is the third most common cancer in women worldwide. Despite organized screening programs, annually 200 women die due to cervical cancer. Current treatments for cervical cancer are platinum based chemotherapy (cisplatin) and radiotherapy, which cause a multitude of DNA damage. To repair this damage, cells possess DNA repair mechanisms. This report will describe the mechanisms involved in repairing DNA damage and analyze whether these mechanisms can be utilized as therapeutic agents to increase efficiency of current treatments of cervical cancer.

Cervical cancer

Cervical cancer is the third most common cancer in women worldwide with an estimated 529,000 new cases in 2008. Approximately 85% of these cases arise in developing countries, where in many countries it is the most common of women cancers (Ferlay et al., 2010).

In developed countries cervical cancer mortality is significantly lower; in the Netherlands incidence and mortality have been steadily declining, with rates of 6.37 and 1.21 per 100,000 women, respectively in 2011 (age-adjusted rates, standardized to the World population) (Integraal Kankercentrum Nederland).

The lower mortality rate in western and northern Europe is attributed to the adoption of organized screening programs (Lowy & Schiller, 2012). Screening in the Netherlands is targeting women between 30 and 60 years old every five years. However, 600–700 women are still diagnosed with cervical cancer each year despite population-wide screening. Annually 200 women die due to cervical cancer (Rogoza et al., 2009).

The three major types of cervical cancer are squamous cell carcinomas (SCC; approx. 80% of cases), adenocarcinomas (AC) and adenosquamous carcinoma (ASC) which comprise approximately 15%.

The major causal factor for cervical cancer is a persistent infection with the human papillomavirus (HPV) showing a close association with the incidence of SCCs. The cofactors that have been associated with an increased risk of acquisition of HPV infection or the progression to cervical cancers include smoking, long-term oral contraceptive use and other sexually transmitted infections (Vaccarella et al., 2013).

HPV type 18 is the predominant risk factor for the development of adenocarcinoma whereas the highly aggressive HPV type16 is associated with both SCC and adenocarcinoma (Bulk et al., 2006). Since cervical cancer has a predominantly viral cause, prevention of this disease through vaccination was considered.

The bivalent HPV-vaccine (Cervarix™) was implemented in 2009 in the Dutch National Immunization Programme (NIP) for 12-year old girls. This vaccine prevents infection with HPV types 16 and 18, which are responsible for approximately 70% of all cervical cancer cases (J. J. Kim & Goldie, 2008).

When cervical cancer does occur, despite the screening and vaccination programs, various treatment regimens exist. Current treatments for advanced stage cervical cancer are platinum based chemotherapy (cisplatin) and radiotherapy (Movva et al., 2009; Serkies & Jassem, 2005).

Although cisplatin is the drug of choice for treatment of a wide variety of tumors, serious side effects are dose-limiting factors (Siddik, 2003). Radiotherapy or surgery, are considered for women with small localized tumors in the cervix whereas for bulky or locally-advanced cervical cancer, the primary treatment is concurrent chemo-radiation with platinum-based chemotherapy (Movva et al., 2009).

Ionizing radiation (IR) can generate a wide variety of DNA breaks such as single-strand breaks (SSBs) and double strand breaks (DSBs). DSBs can also be a result of prolonged replication fork arrest (Petermann et al., 2010; Saintigny et al., 2001) Cisplatin (cis-diammine-dichloroplatinum (II), cDDP, cis-$PtCl_2(NH_3)_2$) introduces covalent links between bases of the same DNA strand (intrastrand crosslinks) or of different DNA strands (interstrand crosslinks or ICLs) and result in replication fork arrest (Hyrien, 2000). This report will describe the mechanisms involved in repairing IR and cisplatin-induced DNA damage and analyze whether these mechanisms can be utilized to increase efficiency of current treatments of cervical cancer.

DNA damage response

To maintain genomic integrity, repair mechanisms have evolved to repair many types of DNA lesions. Mismatch repair (MMR) replaces mispaired bases with the correct bases (Figure 1A). Pyrimidine dimers and intrastrand crosslinks are repaired through nucleotide excision repair (NER; Figure 1B) The base excision repair (BER) pathway (Figure 1C) is activated when small chemical alterations of DNA bases need to be repaired (Lindahl & Barnes, 2000).

Double strand breaks (DSB) are repaired either by homologous recombination (Figure 1D) or by nonhomologous end joining (NHEJ; Figure 1F) (Caldecott, 2008; West, 2003). HR restores the exact genomic sequence by using the sister chromatids as a template, while in NHEJ the ends of the breaks are simply ligated and is prone to generate mutations and deletions (Lieber et al., 2003). Interstrand crosslinks are repaired through the Fanconi anemia pathway (FA; Figure 1E; (Hoeijmakers, 2009; Moldovan & D'Andrea, 2009).

When DNA damage and replication stress are sensed, the DNA damage response (DDR) sets in motion a choreographed response which comprises sensors of DNA damage, recruitment of DNA repair factors, controlling cell cycle checkpoints or initiating apoptosis to protect the cell or ameliorate the threat to the organism (Harper & Elledge, 2007; Jackson & Bartek, 2009).

Sensor proteins recognize DNA lesions and activate the DDR (Zhou & Elledge, 2000) . A next step in the DDR cascade comprises the recruitment of repair factors to sites of DNA damage. In the context of treatment of cervical cancer this report will focus on the DDR pathways following IR (HR and NHEJ pathways) and treatment with cisplatin (activating the NER, FA and the DSB pathways).

To repair DSBs two main strategies are employed; HR and NHEJ. The major pathway to repair DSBs generated by ionizing radiation is NHEJ (Lieber et al., 2003).

The first step in NHEJ is the detection of the DNA ends by the Ku70/80 heterodimer (Figure 1F, top strand); both ends are held in close proximity while at the same time this complex acts as a signal for the recruitment of subsequent NHEJ factors. Ku70/80 is required for the recruitment of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs)(Uematsu et al., 2007).

Interaction with DSB-bound Ku enhances DNA-PKcs' kinase activity (Gottlieb & Jackson, 1993) , which is required for NHEJ (Kurimasa et al., 1999). The second step in NHEJ involves enzymatic processing of DNA ends. IR frequently produces nonligatable DNA ends that must be converted to 3′-hydroxyls and 5′-phosphates before ligation. The factors involved in processing of these DNA structures include aprataxin (APTX), exonuclease 1 (Exo1), Mre11 and Artemis (C. Wang & Lees-Miller, 2013). Processing of DSB ends is predicted to lead to loss or modification of nucleotides from either side of the break, making NHEJ prone to generate mutations and deletions. In the final step of NHEJ (Figure 1F, bottom strand), the DNA ends are re-ligated by DNA ligase IV (LIG4) in a complex with x-ray cross-complementing gene 4 (XRCC4) and XRCC4-like factor (XLF) (Lieber et al., 2003; Mahaney et al., 2009).

In the case of HR, DSBs are recognized by the MRE11-RAD50-Nbs1 (MRN) complex which recruits Ataxia Telangiectasia Mutated (ATM) to the site of the DSB (Bhatti et al., 2011; Petrini & Stracker, 2003; Uziel et al., 2003) (Figure 1D). In undamaged cells ATM exists as a dimer or multimer, is not phosphorylated and is moving free throughout the nucleus. Following DNA damage, ATM becomes a monomer, and phosphorylates other monomers on Ser1981, turning them all into active monomers (Bakkenist & Kastan, 2003). Recruitment of DDR factors involves the ATM-mediated phosphorylation of Ser139 on histone H2AX (Rogakou et al., 1998). Phosphorylated H2AX (γ-H2AX) recruits the MDC1 and initiates an ubiquitination cascade altering the chromatin structure and providing a docking site for RAP80, which ultimately recruits BRCA1-BRCA2-RAD51 repair complex. BRCA1-associated RING domain protein 1 (BARD1) in complex with BRCA1 ubiquinates RAD51 (Wu-Baer et al., 2003) while BRCA 2 interacts directly with RAD51 (Jensen et al., 2010; Thorslund et al., 2010) RAD 51 related proteins Xrcc2 and Xrcc3 are also involved in HR and may contribute to the maintenance of genomic stability (N. Liu et al., 1998).

Besides playing a role in HR, the MRN complex can also initiate NHEJ as the MRE11 subunit can directly interact with the KU70 subunit in the NHEJ pathway (Chapman & Jackson, 2008). Besides the ubiquitination pathway, the accumulation of BRCA1-complex is also dependent on sumoylation (Ciccia & Elledge, 2010).

Because HR relies on a sister chromatid for resection of the DSB, HR is primarily activated in the S/G2 phase of the cell cycle (You & Bailis, 2010) . Inappropriate activation of HR during the G1 phase could lead to cell death, whereas NHEJ activation during the S/G2 phases could increase the chance of mutations. As both HR as well as NHEJ may both be initiated by MRN a regulatory mechanism must take place.

DNA endonuclease RBBP8 (CtIP) is a key regulatory protein that interacts with the MRN complex and ATM (Sartori et al., 2007; You & Bailis, 2010). In the S/G2 phases, phosphorylated CtIP is ubiquitinated by BRCA1, facilitating BRCA1 recruitment to the DSB site. In the G1 phase however, CtIP remains unphosphorilated, leaving the MRN complex inactive as an exonuclease /endonuclease (Huen et al., 2010). NHEJ will then become the dominant repair process.

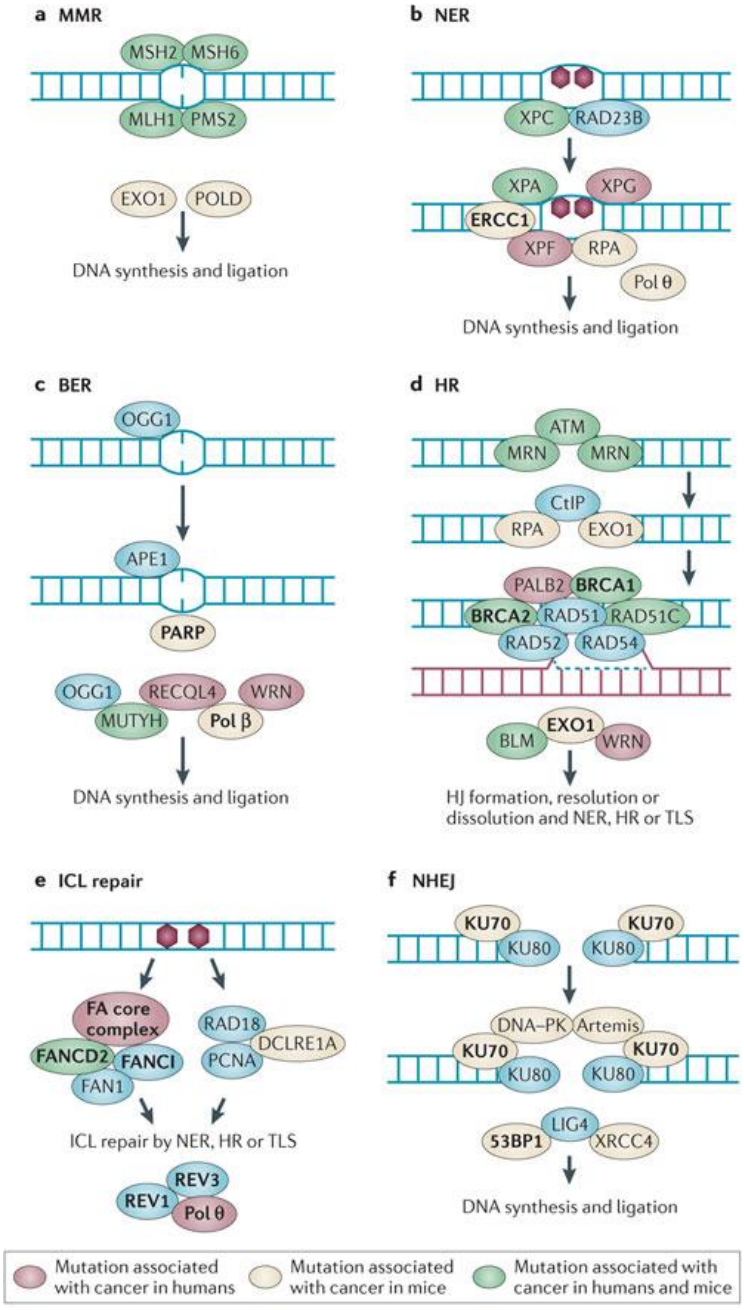

Figure 1: Repair mechanisms have evolved to repair many types of DNA lesions. **(a)** Mismatch repair (MMR) replaces mispaired bases with the correct bases. **(b)** Pyrimidine dimers and intrastrand crosslinks are repaired through nucleotide excision repair (NER), key components are XPA and ERCC1. **(c)** The base excision repair (BER) pathway is activated when small chemical alterations of DNA bases need to be repaired, a key component is PARP. Double strand breaks are repaired either by **(d)** homologous recombination by BRCA1/BRCA2/RAD51 or by **(f)** nonhomologous end joining involving DNA-PK and KU70/80. HR restores the exact genomic sequence by using the sister chromatids as a template, while in NHEJ the ends of the breaks are simply ligated and is prone to generate mutations and deletions. **(e)** Interstrand crosslinks are repaired through the Fanconi anemia pathway. The hexagons represent the DNA lesions, the arrows show the sequence of events (Bouwman & Jonkers, 2012).

Besides ATM, also ATR is also involved in repairing DSBs, however it is single-stranded DNA that is most likely responsible for its activation (Costanzo et al., 2003; Zou & Elledge, 2003). Replication protein A (RPA) coats ssDNA and thereby stabilizes it (Fanning et al., 2006; Wold, 1997). The RPA-ssDNA complex recruits the ATR/ATRIP complex to localize it to the replication fork (Zou & Elledge, 2003). Co-localization of the RAD9-HUS1-RAD1 (9-1-1) complex stimulates ATR kinase activity (Parrilla-Castellar et al., 2004) which results in the activation of CHK1 and CHK2 kinase signaling and phosphorylation of many factors to complete replication (Cimprich & Cortez, 2008).

NER is the main mechanism for removing lesions including cisplatin crosslinks (Figure 1B) (Kartalou & Essigmann, 2001). The XPC–hHR23B complex is the first NER factor to detect a lesion and recruit the rest of the repair machinery to the damaged site in global genome NER (GG–NER) (Sugasawa et al., 1998). The complex has affinity for a variety of g UV-induced injury and by intrastrand crosslinks induced by cisplatin. XPC-hHR23B (Thoma & Vasquez, 2003). At the lesion site XPC–hHR23B recruits TFIIH which opens up an approximately 30-base-long DNA complex around the lesion by utilizing its two helicases XPB and XPD. To stabilize this open intermediate XPA and RPA bind to the nucleotides. XPA binds to the damaged nucleotides while RPA binds to the undamaged DNA strand. Subsequently, XPG, positioned by TFIIH and RPA, makes the 3′ incision. ERCC1–XPF, positioned by RPA and XPA, makes the second incision 5′ of the lesion. The resulting gap is filled in by DNA polymerase δ or ε (Friedberg, 2001; Hoeijmakers, 2001).

The Fanconi anemia (FA) pathway is essential for the repair of DNA interstrand cross-links (Deans & West, 2011; H. Kim & D'Andrea, 2012), a germline mutation in the pathway results in FA (Joenje & Patel, 2001). Almost every protein in the pathway plays a role in at least one other DNA repair pathway as well.

Interstrand crosslinks inhibit the separation of the two DNA strands. During replication in the S phase a stalled replication fork arises and recruitment of FANCM and FAAP24 is initiated, this complex is similar in structure as the ERCC1-XPF complex mentioned in the NER mechanism (Ciccia et al., 2008).

A complex is formed between FANCM-FAAP24 and the histone fold protein 1 (MHF1) which stabilizes the replication fork (Pavri et al., 2006; Yan et al., 2010). Remodeling of the fork by FANCM leads to recruitment of RPA, the single strand DNA-binding protein and subsequent activation of ATR. ATR phosphorylates the MRN complex, FANCD2, and FANCI as well as checkpoint regulator Chk1. As mentioned earlier, the MRN complex associates with CtIP, to regulate HR.

The FANCM-FAAP24 complex also recruits the FA core complex, consisting of eight FA proteins to form an ubiquitin complex, and FANCD2-FANCI (Longerich et al., 2009; Roques et al., 2009). The ubiquitination of FANCD2 and FANCI is a key regulatory step in the FA pathway (Figure 1E). Subsequently nucleases are recruited to the ICL site, using FANCD2 as a landing pad at the ICL site. SLX4-associated MUS81–EME1 and XPF–ERCC1 nucleases cleave the DNA strand and 'unhook' the cross-link (Ciccia et al., 2008). The stalled replication fork is converted to a double-strand break (DSB) and translesion DNA synthesis (TLS) allows the bypass of the unhooked cross-linked oligonucleotides and the restoration of the nascent DNA strand (Lange et al., 2011). The DSB is then repaired by HR, while NER excises the remaining adducts and replication can be re-established.

Cell cycle regulation

The DDR stimulates the recruitment of repair complexes, however it also interacts with proteins required for cell cycle progression resulting in cell-cycle arrest to provide time to repair. Regulation of the cell cycle by the DDR takes place during 3 phases of the cell cycle; the G1/S phase transition, the intra-S phase and the G2/M transition. As mentioned previously, following DSBs, the MRN complex is recruited which activates ATM. ATM plays a key role in cell cycle regulation by phosphorylating the tumor suppressor p53 and checkpoint kinase Chk2. Chk2 subsequently phosphorylates p53 and MDM2 (Bartek et al., 2001; McGowan, 2002). Unphosphorylated MDM2 targets p53 for degradation, Chk2-mediated phosphorylation of MDM2 disrupts its association with p53, thus stabilizing it (Cheng & Chen, 2010). Activation of p53 causes transcription of the cyclin dependent kinase (CDK) inhibitor *p21* (Kastan & Lim, 2000; Wahl & Carr, 2001). This results in the inhibition of Cyclin-E/CDK2 and consequent stalling at the G1/S checkpoint (Kastan & Lim, 2000).

Arrest at the G2/M transition is mediated in several ways. ATM activation causes p53-induced transcription of 14-3-3 σ (Bunz et al., 1998). 14-3-3σ removes cyclinB-cdc2 complex from the nucleus, Cdc2 is required for mitotic entry (Chan et al., 1999). Chk1 inactivates Cdc25c by phosphorylation of Ser 215 and consequent binding of 14-3-3 proteins (Peng et al., 1997). Chk1 also phosphorylates and activates Wee1, which is the kinase that delivers inhibitory phosphorylations on CDKs (Parker & Piwnica-Worms, 1992). Inhibition of Cdc25C prevents the removal of inhibitory phosphates from Cdc2 and therefor also inhibits mitotic entry.

DNA damage response and cervical cancer

The DNA damage response involves checkpoint and repair pathways to facilitate genomic integrity. In the case of cancer it is a dysfunctional DDR mechanism which leads to uncontrolled proliferation.

In several types of cancer somatic mutations in DDR genes have been observed, and for a growing number of DDR genes, hereditary mutations are associated with increased cancer risk. Women with heterozygous germline mutations in *BRCA1* or *BRCA2*, involved in HR (Moynahan et al., 1999; Moynahan et al., 2001), have a 40%–80% risk of developing breast cancer (Fackenthal & Olopade, 2007) . However it has been revealed that *BRCA1* mutation carriers also have a significantly higher risk of developing other cancer types including cervical cancer (Thompson et al., 2002). The relative risk of developing cervical cancer of *BRCA1* mutation carriers compared with non-carriers was 3.72 (Thompson et al., 2002). However, the patient group was relatively small and does not necessarily represent the whole population of cervical cancer patients. Also loss of heterozygosity, resulting in loss of the wild-type allele, has been observed in *BRCA2* mutation carriers in several cancer types including cervical cancer (Gudmundsson et al., 1995). A study of cancer incidence in the relatives of *BRCA1* and *BRCA2* mutation carriers showed a 4.21-fold increased risk of cervical cancer in *BRCA2*-associated families (Johannsson et al., 1999).

Besides mutations, changes in expression of DDR components may also contribute to unlimited proliferation and cancer. Down regulation of tumor suppressor genes and single nucleotide polymorphisms (SNPs) may be involved in susceptibility of the host to cervical cancer.

For example, although the sample size was modest, a statistical association between cervical cancer and XRCC2 R188H polymorphism has been made (Perez et al., 2013). XRCC2 is a RAD51 related protein and is involved in HR (N. Liu et al., 1998). A P72R

polymorphism of p53 has been implicated with cervical cancer in Chinese women (Jiang et al., 2010), however these results could not be replicated in a cohort of Portuguese women (Oliveira et al., 2008). Single-nucleotide polymorphisms in the p21-encoding gene *CDKN1A* codon Arg31Ser seem also to be associated with cervical cancer of Korean women (Roh et al., 2010).

HPV and DDR

The major causal factor for cervical cancer is a persistent infection with HPV. Viral DNA sequences can be detected in 90% of cervical cancers (Patrick et al., 1994) and express the oncoproteins E6 and E7, which may result in chromosomal instability and increased DNA damage during HPV carcinogenesis (Duensing & Munger, 2002; Munger & Howley, 2002).

The normal function of tumor suppressor genes p53 and Rb are inhibited by E6 and E7 proteins (Darnell et al., 2007; Tsai & Chen, 2003) allowing to bypass through at G1 and G2 check points. This data provided solid evidence how HPV16 is causally involved in the development of cervical invasive cancer. E7 also interferes with alternative non-p53 dependent apoptotic pathways (Bosch et al., 2002).

Improving sensitization to radiotherapy or chemotherapy

Faulty DDR mechanisms do not only affect tumor predisposition but also alters the sensitivity of tumors to chemo- and radiotherapy. There is a therefore a broad spectrum of research performed on improving sensitivity of cancer cells to chemo- or radiotherapy.

P53 restoration

The abrogation of functional p53 by E6 and E7 oncoproteins by HPV leads to abrogation of the G1/S cell cycle checkpoint and malignant cell transformation and maintenance of HPV cancer cells. An interesting strategy therefore is the restoration of p53 to induce p53-mediated apoptosis. Research targeting the restoration of p53 in cervical cancer cells has made use of leptomycin B and actinomycin D. Also in this research p53 function was restored with subsequent induction of apoptosis (Hietanen, 1999).

Recent research has shown that celecoxib, a non-steroidal anti-inflammatory drug, stabilizes p53 by downregulating the transcription of E6. Furthermore celecoxib impedes Cox-2 expression (Saha et al., 2012), thereby upregulating p53 transcription de (de Moraes et al., 2007) and inhibiting the COX-2-mediated nuclear localization of p53 (Corcoran et al., 2005; de Moraes et al., 2007; Swamy et al., 2003).

Synthetic lethality

Another strategy to improve the efficiency of radio chemotherapy relies on inducing synthetic lethality. This comprises the inhibition of a functional DDR pathway in cells where other pathways are not functional in repairing DNA damage. A major target is poly(ADP)ribose polymerase (PARP) inhibition in cells with defects of the HR mechanism, especially BRCA1/2 mutations (Bryant et al., 2005; Farmer et al., 2005). PARP is a key player in base excision repair as well as in other DNA repair pathways. When BER as well as HR pathways are inactivated NHEJ becomes activated and contributes to erroneous and possibly lethal repair (Curtin, 2012).

Currently there are several clinical trials using PARP inhibitors, an important inhibitor currently under investigation is AZD2281 (olaparib). Phase 2 clinical trials have been done with patients with breast cancer carrying BRCA1 or BRCA2 mutations which show promising results (Tutt et al., 2010).

At the time of writing the PARP inhibitor olaparib is also the target in a clinical trial targeting women's cancers including cervical cancer. This trial is currently recruiting participants (NCT 01237067) therefore no data is available yet.

Another PARP inhibitor ABT-888 (veliparib) will be used in a clinical trial (NCT01281852) investigating the side effects and the best dose when administered concurrently with treatment using paclitaxel and cisplatin and to see how well they work in treating patients with advanced, persistent, or recurrent cervical cancer, this study is currently also recruiting participants.

Though synthetic lethality has proven an interesting strategy to increase effiency of radiotherapy, there is also evidence that secondary mutations in *BRCA1* and *BRCA2* may actually restore their function, making the cells resistant to PARP inhibitors as well as regaining HR function (Sakai et al., 2008).

However, BRCA-deficient tumors represent only a small fraction of adult cancers including cervical cancers. Therefore other strategies will be needed to induce synthetic lethality. Inhibition of Cdk1 has been suggested as a strategy for using PARP inhibitors in BRCA-proficient cancers. BRCA-wild type cancer cells in which Cdk1 and PARP were both inhibited showed tumor regression with prolonged survival in a mouse model of lung adenocarcinoma. Furthermore, inhibition of Cdk1 did not sensitize non-transformed cells to inhibition of PARP (Johnson et al., 2011). However, results of Cdk1 inhibition in combination with PARP inhibition in cervical cancer cells have not yet been found.

The strategy of synthetic lethality has also been applied to the FA pathway. It has been shown that promoter hypermethylation of *FANCF* gene disrupts the FA-BRCA pathway, resulting in cisplatin resistance (Taniguchi et al., 2003). Loss of expression of a range of FANC genes has furthermore been reported in a variety of cancers, including in 30% of cervical cancer (Narayan et al., 2004). Pharmaceutical inhibition of ATM to induce synthetic lethality may therefor provide the basis for the selective treatment of Fanconi Anemia pathway-deficient cancers providing.

Inhibitors of DDR components

Ever since it was clear that tumor cells have defects in DDR, researches have been searching for inhibitors of key components of the DDR. Early observations showed the radio- and chemosensitizing effects of caffeine were elicited through inhibition of ATM and ATR (Sarkaria et al., 1999).

In vitro analysis showed that cells in which p53 function was inhibited by E6 were able to undergo G1/S and G2/M check point arrest following radiation and that this process relies on active ATM (both G1 and G2) and 53-binding protein 1 (53BP1; G1 only) (Roossink et al., 2012). In this case, the ATM-mediated checkpoint arrest allows DNA repair and cervical tumor cell survival and indicates a poor response to radiation therapy. Inhibition of ATM should therefore result in enhanced sensitivity to radiotherapy. This has already been confirmed *in vitro* using the ATM inhibitor KU-55933 (Hickson et al., 2004) and making use of small interference RNA (siRNA) blocking expression of ATM (Li et al., 2006) in cervical cancer cells.

At time of writing no ATM inhibitors are used in any clinical trials. Another class of inhibitors are phosphatidylinositol 3-kinase related kinase (PIKKs) to which ATM, ATR and DNA-PK belong. The phosphoinositide-3 kinase (PI3K) inhibitors LY294002 and wortmannin have been found to inhibit the PIKKs with differing degrees of activity (Izzard et al., 1999; Sarkaria et al., 1998) and have been under investigation in the context of cervical cancer. Inhibition by LY294002 did also enhance radiation efficiency in cervical

cancer cells (Fuhrman et al., 2008; Y. Liu et al., 2011). However, probably due to their unspecificity, these compounds have not been used in any clinical trials.

The selective DNA-PK inhibitor NU7441 has been shown to act as a chemo sensitizer both *in vitro* and *in vivo* (Zhao et al., 2006). Tumor radiosensitization has also recently been demonstrated *in vitro* and *in vivo* using IC87361, a more selective flavone-based DNA-PK inhibitor (Shinohara et al., 2005). However, selective DNA-PK inhibitors have not yet been reported in the context of cervical cancer.

Similar to cells defective in ATM and ATR, those cells lacking Ku or the DNA-PKcs are sensitive to IR and DSB-inducing chemotherapeutics (Jeggo, 1998). Ku inhibition has been researched in cervical cancer cells, the results of this study showed that indeed Ku70 siRNA could induce sensitize of HeLa cells to radiation therapy (Ayene et al., 2005). Inhibition of Ku80 in HeLa cells also suppressed proliferation *in vitro* and *in vivo* (Zhuang et al., 2007 abstract only).

NER is the main mechanism for removing cisplatin intrastrand crosslinks; therefor the components of NER also make interesting targets. Inhibitors which disturb the interaction of XPA and ERCC1 have been under investigation (Barakat et al., 2012). This research indicated that human colon and lung cancer cell could be sensitized to UV radiation by inhibiting the formation of the XPA-ERCC1 complex using the inhibitors AB-00026258). Earlier studies made use of siRNA against XPA in prostate cancer cells (Cummings et al., 2006). XPA or ERCC1 inhibition has not specifically been assessed in cervical cancer cells, but may be interesting targets to induce chemosensitivity to cisplatin.

Inhibiting Wee1 is an interesting target as cancer cells mostly rely on G2 arrest for DNA damage repair. Wee1 controls G2 by inhibiting phosphorylation of CDC25, inhibiting Wee1 should therefor ablate G2 arrest and result in mitosis entry without repair and subsequent cytotoxic events and apoptosis (Vriend et al., 2013). A requirement of this Wee1 inhibition-mediated radio sensitization is a deficiency in functional p53. As mentioned above, this is the case in cervical cancer through the action of E6 and E7. A highly specific Wee1 inhibitor is MK-1775 has been developed. There is a growing body of evidence that MK-1775 administration causes both *in vitro* and *in vivo* chemosensitization and radiosensitization.

Preclinical research has shown that co-administration of MK-1775 enhanced antitumor efficacy of various classes of DNA damaging agents including cisplatin in p53-deficient cervical (Hirai et al., 2009).

Inhibition of wee1 either by the pyrido-pyrimidine derivative (PD0166285) or via siRNA gene knockdown has also been shown to sensitize various cancer cells including cervical to DNA damage by irradiation (Y. Wang et al., 2001).

Currently a clinical trial studying MK-1775 in combination with cisplatin and radiation in cervical cancer patients is recruiting participants (NCT01925326).

Another compound under investigation is artemisinin. Artemisinin is widely used against malaria and shown to have anticancer properties (Crespo-Ortiz & Wei, 2012). However, the precise molecular mechanism of artemisinin has not yet been elucidated, though generation of radical oxygen species has been implicated (Crespo-Ortiz & Wei, 2012). Artemisinin has a preferentially cytotoxic effect on cervical cancer cells compared to normal cells. It has been shown to sensitize HeLa cells to radiation at a clinically relevant dose. The mechanism of underlying effect of artemisinin involved is thought to be abrogation of the radiation-induced G2 blockade through the restoration

of Wee1 and cyclin B1 expression to the control level (Gong et al., 2012).

Research on UCN-01, an unspecific Chk1 kinase inhibitor has established the therapeutic concept of G2 checkpoint abrogation that potentiates tumor cell killing selectively in p53-deficient tumor cells (Vogel et al., 2007). AZD7762, a Chk1 inhibitor has been studied in Phase 1 clinical trials, however it is unclear whether patients with cervical cancer were included in these studies, which is also the case with LY2603618.

As described above, many strategies to increase the destruction of tumor cells rely on the ablation of cell cycle checkpoint arrests, letting the cells continue with mitosis without the repair mechanisms of the DDR, resulting in mutations, mitotic catastrophe and apoptosis. However, the mutations may also induce further resistance to chemo- or radiotherapy leading the way into a vicious cycle.

Fortunately the evidence presented here does indicate that radiosensitization in cervical cancer cells is possible. However the majority of these positive effects were from *in vitro* experiments. Only a select few chemical agents have progressed to clinical trials to be analyzed for their effects specifically in cervical cancer. Wee1 inhibitor MK-1775 and PARP inhibitor Veliparib will be used in clinical trials for cervical cancer patients and based on pre-clinical data it seems very likely that this will improve sensitivity to chemo- and radiotherapy. However, increasing cross-talk between the different repair pathways is gradually revealed. It seems likely that there will not be a single therapeutic agent effective in all cervical cancer patients. Personalized medicine may become standard practice for the treatment of cervical cancer by assessing the genetic background of the tumor cells and selecting the therapeutic agent with the highest predictive efficiency.

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