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The Molecular Basis of Cisplatin Resistance in Bladder Cancer Cells

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1. Introduction

Bladder cancer is one of the most common cancers among men and women, with men being twice as likely affected from the disease (Jemal et al., 2005). The most common type of bladder cancer is transitional cell carcinoma (TCC), which is derived from the urothelium and constitutes more than 90 % of all bladder cancers (Bischoff & Clark, 2009). Cisplatin-based combination therapy is the standard therapy for the treatment of advanced or metastatic bladder cancers (Cohen et al., 2006, Kaufman, 2006). However, the outcome of patients with metastatic bladder cancer remains poor, as tumors become resistant to cisplatin therapy. It is still not entirely known, which factors influence the response of bladder cancers to the drug and how this cancer acquires cisplatin resistance. Cisplatin is a neutral planar complex (Figure 1A).
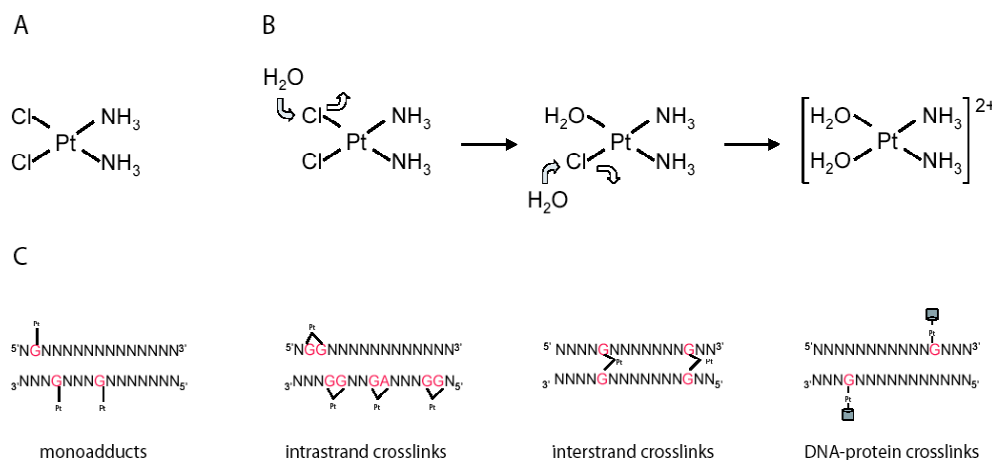

Fig. 1. **A:** The chemical structure of cisplatin. **B:** After entering the cells, cisplatin is transformed to a positively charged molecule that reacts with DNA **C:** Cisplatin induced lesions. Cisplatin preferably binds to the nucleophilic N7 position of the purine bases guanine or adenine, leading to different types of lesions including monoadducts, intrastrand crosslinks, interstrand crosslinks and DNA-protein crosslinks

After entering the cell, it is activated through a series of aquation reactions, in which the chloro ligands are replaced by water molecules (Figure 1B). The resulting positively charged molecule can react with nucleophilic sites on macromolecules, leading to DNA, RNA and protein adducts. It preferably binds to the nucleophilic N7 position of the purine bases guanine or adenine, which leads to different types of lesions (Figure 1C) (Jamieson & Lippard, 1999). In a first reaction, cisplatin binds to DNA, leading to monoadducts, which in a second reaction lead to the formation of DNA crosslinks. The most frequently observed cisplatin DNA lesions are DNA intrastrand crosslinks between adjacent guanines (65 % of all lesions) or intrastrand crosslinks between guanine and adenine (25 %). Interstrand crosslinks between two guanines on the opposite strands of DNA account for less than 5% of all cisplatin-induced lesions. It is still unknown, which of the various DNA lesions ultimately results in cell death (Chu, 1994, Jordan & Carmo-Fonseca, 2000, Kartalou & Essigmann, 2001).

The efficacy of cisplatin in cancer chemotherapy, however, is limited by resistance. While cancers of the bladder, lung and ovary respond initially in 50 % or more of cases, they will almost inevitably relapse with drug-resistant disease. The mechanisms of cisplatin resistance have been studied in numerous cell culture models of cisplatin sensitive and resistant cancer cells lines. It has been shown that a cancer cell can develop cisplatin resistance through different mechanisms (Figure 2). Cisplatin resistance can be due to (i) changes in drug transport, leading to reduced cellular cisplatin accumulation, (ii) increased drug detoxification, also resulting in reduced cellular cisplatin accumulation, (iii) changes in DNA repair mechanisms including nucleotide excision repair, interstrand crosslink repair and mismatch repair, (iv) changes in DNA tolerance mechanisms, and finally (v) alterations in the apoptotic cell death pathways (Köberle et al., 2010, Rabik & Dolan, 2007, Siddik, 2003). In this chapter we describe and discuss the contribution of these mechanisms for the development of cisplatin resistance in bladder cancer cells *in vitro* and compare the preclinical findings to data obtained in clinical studies. A better understanding of the molecular basis of cisplatin resistance may lead to new anticancer strategies that will sensitize unresponsive bladder cancers to cisplatin-based chemotherapy.
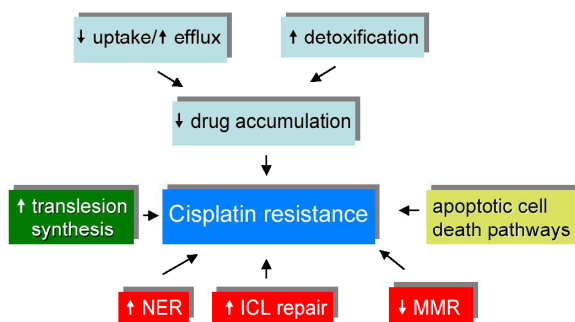

Fig. 2. Mechanisms of resistance towards cisplatin include: Reduced drug accumulation due to changes in drug uptake, efflux or detoxification. Alterations in DNA repair such as increased removal of the damage by nucleotide excision repair (NER) or interstrand crosslink repair (ICL repair) as well as decreased mismatch repair (MMR). Enhanced translesion synthesis (TLS) to tolerate unrepaired cisplatin lesions. Alterations in apoptosis pathways: changes in expression levels of pro- and anti-apoptotic proteins.

2. Intracellular drug accumulation as a determinant of cellular cisplatin sensitivity

2.1 Cellular uptake and efflux of cisplatin

Reduced intracellular cisplatin accumulation has been associated with cisplatin resistance in numerous cancer cell lines (Siddik, 2003). A correlation between intracellular cisplatin accumulation and cisplatin resistance was observed in a series of seven bladder cancer cell lines displaying different sensitivities to cisplatin (Koga et al., 2000). Similarly, using a bladder cancer cell line and its cisplatin-resistant subline, we found reduced accumulation of cisplatin in the resistant subline when compared to its parental cells (Köberle et al., 1996). Reduced accumulation may result from changes in drug transport or increased drug detoxification. Even though the exact mechanism by which cisplatin is taken up by the cells is not fully understood, both passive diffusion and active transport appear to be involved. For active transport the copper transporter 1 (Ctr1), which controls intracellular copper homeostasis, seems to play an important role (Kuo et al., 2007, Safaei, 2006). It has been reported that loss of Ctr1 lead to cisplatin resistance in various cell lines (Holzer et al., 2006, Ishida et al., 2002, Song et al., 2004). However, no data as to Ctr1 expression in bladder cancer cell lines or tumor tissue have been reported to date. Therefore, no conclusion about the importance of uptake for cisplatin response can be drawn for bladder cancer cells (Table 1).

Increased efflux of cisplatin from the cell may also lead to resistance. Efflux pumps such as MRP1/2 (multidrug resistance associated protein) and p-glycoprotein/multidrug resistance 1 (MDR1) are implicated as efflux pumps for cisplatin (Taniguchi et al., 1996, Yeh et al., 2005). Tada and co-workers investigated the relationship between expression of p-glycoprotein expression or MRP1/2 and drug sensitivity in 47 clinical samples of bladder cancer. They showed that expression of p-glycoprotein and MRP1/2 was higher in samples of recurrent tumors than in untreated primary tumors (Tada et al., 2002), indicating that increased efflux can contribute to the development of drug resistance and poor clinical outcome in bladder cancers (Table 1).

2.2 Detoxification of cisplatin by intracellular thiol molecules

Cisplatin resistance can be the result of increased inactivation of the drug by intracellular thiol-containing molecules such as glutathione and metallothionein. Glutathione is a tripeptide that plays an important role for the detoxification of xenobiotic substances by scavenging free radicals. Cisplatin can be conjugated with glutathione, which will inhibit its binding to DNA and other cellular molecules. This reaction is catalyzed by the glutathione-S-transferase (GST) (Mannervik, 1987). Extensive studies about the role of the glutathione system for cisplatin resistance have been carried out in cell lines and in cancer tissue. A correlation between expression of the glutathione system and cisplatin resistance has been reported for ovarian, cervical and lung cancer cell lines (Jansen et al., 2002, Meijer et al., 1992, Mellish et al., 1993). Attempts to correlate expression of the glutathione system with cisplatin resistance in bladder cancer cell lines showed inconsistent findings. Bedford and co-workers investigated the expression of the glutatione system in different bladder cancer cells lines and reported higher levels of glutathione and GST in the less sensitive cells (Bedford et al., 1987). Similarly, using a model system of a bladder cancer cell line and two derived sublines with acquired cisplatin resistance, Kotoh and co-workers observed an increased glutathione content and elevated GST activity in the sublines (Kotoh et al., 1997). Buthionine sulphoximine (BSO), which depletes glutathione, or indomethacin, which blocks

GST, significantly decreased the cisplatin resistance in T24 bladder cancer cells, which is yet another indication that the glutathione-based detoxification system is involved in cisplatin resistance in bladder cancer cells (Byun et al., 2005). However, no correlation between glutathione content and resistance to cisplatin was observed in a study by Koga and co-workers (Koga et al., 2000). In this study, the expression of GST was also not significantly related to cisplatin resistance. In another study with bladder cancer cells, which were either sensitive or progressively resistant to cisplatin, it was observed that expression of GST was increased in the cisplatin resistant cells, however, the increase in glutathione contents did not reach statistical significance (Hour et al., 2000). In conclusion, bladder cancer cells may gain cisplatin resistance through up-regulation of GST, while glutathione contents seems to play a less important role for the development of cisplatin resistance.

Metallothioneins (MT) belong to a family of low molecular weight, thiol-rich proteins that play a role in metal homeostasis and detoxification (Kagi & Schaffer, 1988). MTs can bind to cisplatin, leading to the inactivation of the drug. For numerous cancer cell lines (derived from prostate, lung, ovary and cervical cancer), a correlation between MT expression and cisplatin resistance has been observed (Kasahara et al., 1991, Kondo et al., 1995, Mellish et al., 1993, Surowiak et al., 2007). For bladder cancer cell lines cisplatin resistance, was also correlated with increased levels of MT (Siegsmund et al., 1999, Singh et al., 1995). A role of MT for cisplatin resistance in bladder cancer has been proposed by Satoh and co-workers (Satoh et al., 1994). The authors investigated the effect of modulation of the MT levels for the antitumor activity of cisplatin in nude mice inoculated with human bladder cancer cells. While increasing MT levels reduced the antitumor activity of cisplatin, decreased levels of MT diminished the resistance to the drug (Satoh et al., 1994). Using a different bladder tumor model in mice, it was also suggested that MT might play a role for acquired resistance towards cisplatin (Saga et al., 2004). The clinical relevance of MT levels for cisplatin chemotherapy in bladder cancers has been investigated in a number of studies. In an investigation involving 118 patients with bladder cancer, it was observed that overexpression of MT was associated with a poorer outcome from cisplatin-based chemotherapy (Siu et al., 1998). Similarly, for intrinsic cisplatin resistance of urinary tract TCCs, an involvement of MT has been suggested (Kotoh et al., 1994), and MT overexpression was proposed to be a mechanism for cisplatin resistance in bladder cancer tissue (Wood et al., 1993). In line with this observations are more recent studies, which also reported that high levels of MT expression in bladder cancer tissue were correlated with poor survival after cisplatin chemotherapy (Hinkel et al., 2008, Wülfing et al., 2007). Taken together, the data indicate that high levels of MT in bladder cancers might be a major problem for effective cisplatin-based chemotherapy. In our opinion, expression of MT is one of the main cellular factors for both intrinsic and acquired cisplatin resistance in bladder cancers (Table 1).

3. DNA repair and cisplatin resistance

The contribution of DNA repair for cisplatin resistance has been investigated for many years. In model systems of tumor cell lines and sublines with acquired cisplatin resistance, increased removal of cisplatin induced lesions has been observed in the sublines. For example, ovarian cancer cells with acquired resistance towards cisplatin show an increased removal of cisplatin induced lesions in comparison with their cisplatin sensitive counterparts (Johnson et al., 1994a, Johnson et al., 1994b, Parker et al., 1991). Similarly, colon

Molecular mechanism	Preclinical evidence	Clinical evidence
Intracellular cisplatin accumulation		
Decreased uptake	no data reported	no data reported
Increased efflux	no data reported	observed in resistant tumors
Increased glutathione/ GST levels	conflicting results	Conflicting results
Increased metallothioneine levels	Observed in resistant cancer cell lines	correlated with poor clinical outcome
DNA repair		
Nucleotide excision repair (NER)	High levels of ERCC1 in resistant cancer cells	Conflicting results
ICL repair	Proficiency in resistant bladder cancer cell lines	no data reported
Translesion synthesis (TLS)	no data reported	no data reported
Mismatch repair (MMR)	No association with acquired resistance	Conflicting results
DNA damage response		
p53	Conflicting results	Conflicting results
Bcl-2, Bcl-xL	Overexpression in cisplatin resistant cancer cells	Low levels correlate with better prognosis
Survivin	High levels in bladder cancer cells	Expression as a marker for clinical outcome
XIAP	High expression in bladder cancer cells	High levels correlate with poor prognosis

Table 1. Mechanisms of cisplatin resistance in bladder cancers: preclinical findings and clinical evidence (Table adapted from Köberle et al., 2010)

carcinoma cell lines with acquired cisplatin resistance showed a higher extent of removal of DNA platination compared to the parental cells (Oldenburg et al., 1994), indicating that the

acquired resistance to cisplatin might be related to the increased DNA repair capacity. In contrast, when we investigated DNA damage removal in a bladder cancer cell line with acquired cisplatin resistance, we observed no enhanced repair compared to the parental cell line, suggesting that this bladder cancer cell line did not acquire resistance to cisplatin by increasing the DNA repair capacity (Köberle et al., 1996). However, when we compared bladder cancer cell lines with cisplatin sensitive testis tumor cells, we observed that bladder cancer cells are proficient in removing cisplatin damage from the DNA, while testis tumor cells were repair deficient (Köberle et al., 1997), supporting the hypothesis that susceptibility to cisplatin might be related to the repair capacity.

3.1 Nucleotide excision repair

Cisplatin-induced GpG and GpA DNA intrastrand crosslinks are repaired by nucleotide excision repair (NER). NER is a multistep mechanism, which deals with bulky helix-distorting lesions such as UV-induced cyclobutane pyrimidine dimers and 6-4 photoproducts, and DNA lesions induced by many chemotherapeutic drugs (Gillet & Schärer, 2006, Shuck et al., 2008, Wood et al., 2000). The repair of the lesions begins with recognition of the damage and incision on both sides of the lesion, followed by DNA synthesis to replace the excised fragment. The core incision reaction requires the protein factors XPA, RPA, XPC-HR23B, TFIIH, ERCC1-XPF and XPG (Aboussekhra et al., 1995). It is possible to carry out the core NER reaction in a cell free system using cellular protein extracts (Shivji et al., 1999, Shivji et al., 2005). Using this system, it could be confirmed that the increased removal of cisplatin lesions, which has been observed in cisplatin resistant ovarian cancer cells, is in fact due to enhanced NER (Ferry et al., 2000). We found that cellular protein extracts of a bladder cancer cell line were proficient for NER (Köberle et al., 1999). Furthermore, the core NER proteins are expressed to a similar extent in bladder cancer cell lines compared to normal non-cancerous cells (Köberle et al., 1999, Welsh et al., 2004). The removal of cisplatin induced DNA platination, which we previously observed in bladder cancer cell lines (Köberle et al., 1997), is therefore, at least in part, due to NER proficiency in these cells.

Conclusive evidence for functionally increased NER in cisplatin-resistant cancers, however, has not yet been presented. This is due to the lack of methods to easily and reliably measure NER activities in tissue samples. For example, even in protein extracts prepared from cell lines, a significant variability in NER capacity is observed. Even more, in protein extracts prepared from biopsies of human ovarian carcinoma, Jones and co-workers found that the NER capacity varied significantly by as much as ten-fold (Jones et al., 1994). This could be due to either inter-individual variations or to technical problems to obtain active extracts from tissue material. Therefore, as measuring NER capacity in tissue samples is a challenging task, a different approach is to investigate the expression of NER factors on the mRNA or protein level and attempt to correlate these with response to chemotherapy. In these studies, special emphasis was given to ERCC1, the first human DNA repair gene cloned (Westerveld et al., 1984). In preclinical studies, a correlation between ERCC1 expression and cisplatin resistance has been presented (Li et al., 1998, Li et al., 2000, Metzger et al., 1998). By demonstrating that down-regulation of ERCC1 by siRNA sensitized bladder cancer cell lines to cisplatin, we could confirm the importance of ERCC1 for cisplatin resistance in bladder cancer cells (Usanova et al., 2010).

In cancer tissues, ERCC1 mRNA or protein levels show an inverse correlation with the response to platinum therapy or overall survival. High ERCC1 mRNA levels are associated with resistance to cisplatin-based chemotherapy in ovarian, cervical, gastric, colorectal, head and neck, esophageal and lung cancer (Dabholkar et al., 1992, Dabholkar et al., 1994, Gossage & Madhusudan, 2007, Handra-Luca et al., 2007, Jun et al., 2008, Kim et al., 2008, Metzger et al., 1998, Olaussen et al., 2006, Weberpals et al., 2009). Based on these findings, it was suggested that ERCC1 can be used as a predictive and prognostic marker for the outcome of cisplatin-based chemotherapy. For patients with advanced bladder cancer, a significantly higher survival rate was reported when ERCC1 levels in the tumor tissue were low (Bellmunt et al., 2007). However, in another study, no significant difference in overall survival between bladder cancer patients with ERCC1 negative tumors and ERCC1 positive tumors was observed (Kim et al., 2010). On the other hand, the authors reported that progression free survival was longer in patients with ERCC1 negative bladder cancers compared to ERCC1 positive cancers (Kim et al., 2010). Based on these conflicting results, it is difficult to conclude that ERCC1 expression in bladder cancer negatively contributes to the clinical outcome. Furthermore, even though ERCC1 positive tumors would be expected to have a high NER capacity, and ERCC1 negative tumors would be expected to have low NER capacity, these conclusions must be drawn with caution, as functional NER assays for tissue material are still missing. It therefore remains speculative whether altered ERCC1 levels have an impact on NER in tumor tissue. Therefore, the question about the contribution of enhanced NER for cisplatin resistance in cancers, especially in bladder cancers, remains to be solved (Table 1).

3.2 Interstrand crosslink repair

Besides intrastrand adducts, cisplatin induces interstrand crosslinks (ICLs), which are removed by ICL repair, a process less understood than NER (McHugh et al., 2001). Repair of ICLs is a challenging problem for cells. In bacteria and lower eukaryotes, NER and homologous recombination are involved in ICL repair (Cole, 1973, Jachymczyk et al., 1981). In mammalian cells, these both pathways may also operate (De Silva et al., 2000). Besides that, mammalian cells have additional pathways of ICL repair involving DNA polymerases that can bypass the lesion (Sarkar et al., 2006, Shen et al., 2006, Zheng et al., 2005). A contribution of increased ICL repair for acquired resistance to cisplatin has been described for ovarian cancer cells in culture (Zhen et al., 1992). It also seems to play a role for clinical cisplatin resistance, as in paired tumor samples obtained prior to treatment and at relapse following platinum chemotherapy, increased repair of cisplatin ICLs in cells of relapsed ovarian cancer was observed (Wynne et al., 2007). We found that bladder cancer cell lines, which are relatively resistant to cisplatin, are proficient in repairing ICLs (Usanova et al., 2010). Biochemical and cell biological data implicate that ERCC1 is not only involved in NER, but also in ICL repair (Kuraoka et al., 2000, Niedernhofer et al., 2004, Sijbers et al., 1996). Our own experiments revealed that down-regulation of ERCC1 by siRNA affected ICL repair in the bladder cancer cell lines and rendered the cells more sensitive to cisplatin supporting the notion about the importance of ICL repair for cisplatin resistance in cancer cells. However, to date there is no information as to ICL repair in bladder cancer tissue (Table 1).

3.3 Translesion synthesis (TLS)

As described in 3.1 and 3.2, cisplatin damage is removed by NER and ICL repair. However, some lesions may remain. A mechanism, by which cells can tolerate unrepaired DNA

lesions, is translesion synthesis (TLS). TLS is carried out by a group of specialized DNA polymerases, which are capable of bypassing unrepaired DNA lesions. For mammalian cells pol η (POLH), pol ι (POLI), pol κ (POLK), REV1 and pol ζ (REV3 and REV7) are the main TLS polymerases, which have been shown to possess different substrate specificity. Depending on the type of damage, different combinations of TLS polymerases act in concert to bypass the DNA lesions (Shachar et al., 2009). Cisplatin GpG intrastrand crosslinks seem to be bypassed by pol η and pol ζ (Alt et al., 2007, Shachar et al., 2009). For pol κ conflicting results have been reported. While an in vitro assay suggests that pol κ is unable to bypass a GpG intrastrand crosslink, in vivo TLS assays implicated pol κ in combination with pol η for TLS across cisplatin GpG intrastrand crosslinks (Ohashi et al., 2000, Shachar et al., 2009). The importance of TLS in the tolerance towards cisplatin has been shown in cell lines deficient in TLS polymerase activity (Cruet-Hennequart et al., 2008, Cruet-Hennequart et al., 2009, Albertella et al., 2005a, Roos et al., 2009, Wittschieben et al., 2006). Similarly, TLS polymerases may play a role for cisplatin resistance in tumor samples (Albertella et al., 2005b, Ceppi et al., 2009, Wang et al., 2009). However, no data have been reported as to the expression of TLS polymerases in bladder cancer cell lines and tumor specimens. We therefore can neither include nor exclude TLS polymerases as a factor determining efficacy of cisplatin therapy in the clinic (Table 1).

3.4 DNA mismatch repair (MMR)

Mismatch repair (MMR) is the pathway that removes mispaired nucleotides or insertion/deletion loops, which arise during DNA replication or as a result of damage to DNA. MMR consists of following steps: (1) recognition of the mismatch, (2) identification and excision of the mispairs or looped intermediates, and (3) resynthesis of the excised strand (Kunkel & Erie, 2005). In early investigations it has been observed that loss of MMR led to resistance to cisplatin and other platinating agents (Aebi et al., 1996, Fink et al., 1996). A possible explanation for the association of absence of a repair mechanism with increased drug resistance was the observation that MMR proteins can bind to cisplatin damage possibly leading to futile repair and therefore increased drug lethality. The mismatch repair complex MutSα (which is a heterodimer containing MSH2 and MSH6) binds to cisplatin DNA lesions in vitro (Duckett et al., 1996, Mello et al., 1996). Binding of MutSα to cisplatin crosslinks could start the MMR process by recruiting the mismatch repair complex MutLα (consisting of MLH1 and PMS2). It is assumed that lethal intermediates arise by the attempt of the MMR machinery to remove cisplatin lesions, and these lethal intermediates might set off a futile MMR cycle, similar to what has been reported for methylating agents (Dunkern et al., 2001). An alternative model suggests that binding of the MMR complex to cisplatin DNA damage might cause direct activation of the DNA damage response (DDR). A third model is based on the finding that TLS polymerases can bypass of 1,2-intrastrand crosslinks (Alt et al., 2007, Shachar et al., 2009). Since TLS polymerases are error prone causing mis-incorporation of bases, mismatches will be generated that are recognised by the MutSα complex. This in turn causes a futile repair cycle that triggers DDR. New data suggest that mitochondrial pro-death signaling involving cytochrome c and caspases-9 and -3 is required for the execution of MMR protein-mediated induction of cell death by cisplatin (Topping et al., 2009). The importance of MMR for cisplatin resistance has been investigated in a number of cancer cell lines, however, with conflicting results. On the one hand it was observed that

MMR deficient cell lines were more tolerant to cisplatin (Bignami et al., 2003, Fink et al., 1996, Papouli et al., 2004). This was explained by the hypothesis that cisplatin lesions are not processed into lethal intermediates. In other studies, however, it was shown that defective MMR is only a minor contributor for the cisplatin resistance phenotype or is not involved at all (Branch et al., 2000, Claij & te Riele, 2004, Massey et al., 2003). We found that the MMR protein MSH2 was expressed at lower levels in bladder cancer cells compared to cisplatin sensitive testis tumor cells. However, no difference was observed in the expression level of the MMR proteins hMLH1 and PMS2 in this model system of cisplatin resistant and sensitive cell lines. Even more, no difference in the levels of MSH2, MLH1 and PMS2 was observed in parental RT112 bladder cancer cells and the subline with acquired cisplatin resistance (Köberle, unpublished results), suggesting that MMR may not be of importance for cisplatin resistance in our model system.

The clinical relevance of loss of MMR for cisplatin chemotherapy has been investigated in a number of clinical studies, and it was concluded that MMR deficiency is associated with chemotherapy resistance in ovarian and testicular germ cell tumors (Gifford et al., 2004, Helleman et al., 2006, Wei et al., 2002). In 115 patients with bladder cancers, the expression pattern of hMSH2 protein was investigated and a reduced expression of hMSH2 was significantly more frequent in high grade tumors (Jin et al., 1999). Similarly, Catto and co-workers reported that reduced expression of hMLH1 and hMSH2 was seen more commonly in muscle invasive and high grade bladder cancer (Catto et al., 2003). In contrast, in a set of 130 urothelial carcinomas of the bladder, hMSH2 and hMSH6 negative tumors were found to have a favorable impact on overall patient survival (Mylona et al., 2008). In a number of studies, the degree of microsatellite instability (MSI) was investigated in different cancer tissues, such as colorectal-, ovarian- and gastric carcinoma (Dietmaier et al., 1997, Ichikawa et al., 1999, Ottini et al., 1997). MSI is the result from inactivating mutations in MMR genes and suggests MMR deficiency (Parsons et al., 1993, Strand et al., 1993). However, MSI has been observed only infrequently in bladder cancer tissues (Bonnal et al., 2000, Gonzalez-Zulueta et al., 1993, Hartmann et al., 2002). Furthermore, reduced expression of hMLH1 and hMSH2 was not correlated with MSI in bladder cancer (Catto et al., 2003). Based on these conflicting data, a conclusion as to whether MMR impacts the development of cisplatin resistance in bladder cancer in the clinic cannot be drawn to date (Table 1).

4. DNA damage response and apoptosis pathways in cisplatin resistance

It is known that cisplatin treatment induces apoptosis in cells, thereby killing the cells (Chu, 1994). The apoptotic pathways, which are induced following cisplatin treatment, were extensively studied, hence not yet fully understood. Cisplatin-induced apoptosis may be triggered through the extrinsic death receptor pathway, which is mediated through the JNK signaling cascade. Alternatively, the intrinsic mitochondrial pathway may be induced, mediated through p53 and anti- or pro-apoptotic members of the Bcl-2 family proteins (Brozovic et al., 2004, Pabla et al., 2008, Siddik, 2003). Decreased expression or loss of pro-apoptotic proteins may result in cisplatin resistance, similarly may increased expression of anti-apoptotic proteins lead to cisplatin resistance (Brozovic & Osmak, 2007). The contribution of these mechanisms for preclinical and clinical cisplatin resistance of bladder cancer cells will be discussed in the following section.

4.1 p53 and cisplatin resistance of bladder cancer cells

The tumor suppressor protein p53 is activated in cancer cells after treatment with chemotherapeutic drugs and has a central role for the induction of apoptosis. The influence of the p53 status for cisplatin resistance has been studied in numerous cancer cell lines, however, with contradictory results. While no correlation between cisplatin resistance and p53 status was observed in testis and ovarian cancer cell lines (Burger et al., 1997, De Feudis et al., 1997), other studies using breast, lung, colon, kidney, ovarian, leukaemia, melanoma and prostate cancer cell lines showed that p53 mutated cell lines were more resistant to cisplatin compared to p53 wild-type cell lines (Branch et al., 2000, O'Connor et al., 1997).

Contradictory results about the importance of p53 status for cisplatin resistance are also reported for bladder cancer cells. Comparing the cisplatin sensitivity in bladder cancer cell lines with different p53 status revealed that p53 wild type bladder cancer cells were more susceptible to cisplatin, while mutant cell lines were resistant (Kawasaki et al., 1996, Konstantakou et al., 2009). In line with these findings, it was also shown that cisplatin resistance in bladder cancer cells was enhanced by overexpression of mutant p53 protein (Miyake et al., 1999). Our own studies revealed that cisplatin resistant bladder cancer cell lines were mutated for p53, while cisplatin sensitive testis tumor cells showed functional p53 activity after cisplatin treatment (unpublished results). Contrary to these observations, Chang and co-workers investigated the effect of p53 mutations for drug sensitivity and found that bladder cancer cell lines expressing various human mutated p53 proteins displayed enhanced cisplatin sensitivity (Chang & Lai, 2001). Even more, when cisplatin sensitivity was measured in a series using 89 bladder cancer cell lines with different p53 status, it was found that p53 heterozygous cells were most susceptible to cisplatin (Chang & Lai, 2000). Altogether, we therefore conclude that, at least in bladder cancer cell lines, p53 mutations do not always lead to the development of cisplatin resistance.

In a number of studies it has been investigated whether the p53 status can be a predictor for the response to platinum-based chemotherapy in the clinic. Gadducci and co-workers reported that ovarian cancer patients with tumors harbouring p53 mutations experience a lower chance to achieve a complete response following cisplatin therapy, while patients with wild-type p53 tumors have a good chance to respond (Gadducci et al., 2002). In bladder cancers, mutations in the p53 gene are a frequent event (Esrig et al., 1994). However, there are conflicting results whether the p53 status can be used to predict the responsiveness to cisplatin treatment in bladder cancers (Nishiyama et al., 2008). On the one hand, it was shown that in a cohort of patients with TCC only the patients with altered p53 in the tumor would benefit from adjuvant cisplatin chemotherapy (Cote et al., 1997). On the other hand, p53 immunoreactivity could not be used to predict tumor response and patient survival in a cohort of 83 patients (Qureshi et al., 1999). Similarly, no clear conclusion as to whether p53 wild type was related to increased resistance or increased responsiveness could be drawn by Watanabe and co-workers in a study investigating 75 tumor specimens (Watanabe et al., 2004). Therefore, it cannot be concluded to date that the p53 status influences cisplatin responsiveness in bladder cancers (Table 1).

4.2 Anti-apoptotic proteins and cisplatin resistance

Cisplatin resistance has been associated with the expression of a number of anti-apoptotic proteins, both in cell cultures and in clinical samples. Expression of the anti-apoptotic proteins Bcl-2 and Bcl-x_L resulted in cisplatin resistance in ovarian cancer cell lines (Yang et

al., 2004). In bladder cancer cell lines, which were resistant to cisplatin and etoposide, Chresta and co-workers also observed high levels of Bcl-2 (Chresta et al., 1996). In addition, levels of the pro-apoptotic protein Bax were very low in the three bladder cancer cell lines under investigation (Chresta et al., 1996). We also observed low endogenous levels of Bax in cisplatin resistant bladder cancer cells compared to cisplatin sensitive testis tumor cell lines (unpublished observations). Furthermore, cisplatin treatment lead to translocation of Bax to the mitochondrial membrane in testis tumor cells, which was not observed in bladder cancer cell lines (unpublished observations). An association between cisplatin resistance, Bcl-2 expression and Bax translocation has also been proposed by Cho and co-workers who observed in cisplatin resistant bladder cancer sublines that Bcl-2 was up-regulated, which resulted in inhibition of Bax translocation to the mitochondrial membrane and reduced cell death (Cho et al., 2006). To elucidate the role of Bcl-2 for cisplatin resistance in bladder cancer cells, Miake and co-workers transfected the human bladder transitional cell carcinoma line KoTTC-1 with an expression plasmid for Bcl-2 and observed that overexpression conferred resistance to cisplatin (Miyake et al., 1998). Stably expressing Bcl-2 cells were then injected subcutaneously into nude mice to determine whether the Bcl-2 status can affect the efficacy of cisplatin treatment. Using this tumor cell implantation model, the authors could show that mice with tumors expressing Bcl-2 have an inferior prognosis compared to mice with tumors with no detectable Bcl-2 protein (Miyake et al., 1998). Altogether, the data suggest that Bcl-2 might be one of the factors influencing cisplatin resistance in bladder cancer cells. In proof of principle experiments, Bcl-2 levels in bladder cancer cells were decreased using Bcl-2 antisense oligonucleotides. These studies revealed that down-regulation of Bcl-2 expression resulted in a significant increase in toxicity of cisplatin in various bladder cancer cell lines (Bolenz et al., 2007, Hong et al., 2002), supporting the notion that expression of Bcl-2 may be associated with cisplatin resistance in bladder cancer cells (Table 1).

Expression levels of the anti-apoptotic factors Bcl-2 and Bcl-xL were determined in tumor samples from a diverse range of tissue to investigate for a possible involvement in clinical resistance, however, with contradictory results. While in ovarian carcinoma patients, expression of Bcl-xL was correlated with a decreased response to platinum chemotherapy (Williams et al., 2005), no association between response and Bcl-2 expression was observed in breast cancer patients (Parton et al., 2002). For bladder cancers, the clinical relevance of Bcl-2 expression for cisplatin resistance has been shown by Cooke and co-workers. The authors observed in a cohort of 51 patients with bladder cell carcinoma who received neo-adjuvant cisplatin chemotherapy that patients with Bcl-2 negative tumors had a significantly better prognosis (Cooke et al., 2000). An improved survival of patients with Bcl-2 negative tumors was also observed in a cohort of 89 patients with invasive bladder cancers who received cisplatin-based chemotherapy (Kong et al., 1998). In conclusion, expression of the anti-apoptotic factor Bcl-2 appears to affect the efficacy of cisplatin therapy for bladder cancers and might be used as a prognostic marker to predict the response to treatment.

The inhibitor of apoptosis (IAP) gene family encodes proteins, which have been reported to play an important role in cellular drug resistance. These proteins have been shown to be endogenous inhibitors of caspases, thus resulting in inhibition of cell death. Survivin, one of the members of the IAP family, is activated by cisplatin, which in part protects cells from cisplatin-induced apoptosis (Belyanskaya et al., 2005). An associated between survivin levels and cisplatin resistance has been reported for a number of cell lines derived from various cancer tissues including thyroid, lung and colon (Tirro et al., 2006) (Belyanskaya et al., 2005,

Hopkins-Donaldson et al., 2006, Pani et al., 2007). Bladder cancer cell lines showed a high expression of survivin compared to non-cancerous uro-epithelial cells (Yang et al., 2010).

In clinical studies it has been investigated whether survivin might serve as a prognostic marker to predict clinical outcome. In tumor material of 30 patients with advanced bladder cancer, survivin expression has been identified as a marker for poor clinical outcome (Als et al., 2007). Similarly, Shariat and co-workers identified survivin as an independent predictor for recurrence of the disease in a cohort of 726 patients (Shariat et al., 2009).

The X-linked inhibitor of apoptosis (XIAP) is another member of the family of IAP proteins. Preclinical studies indicate that XIAP expression may be associated with cisplatin resistance. In ovarian carcinoma cell lines, for example, enhanced expression of XIAP was connected to the acquisition of cisplatin resistance (Mansouri et al., 2003). Bilim and co-workers reported considerable levels of XIAP in a panel of 4 bladder cancer cell lines, which are known to be cisplatin resistant (Bilim et al., 2003). The clinical relevance of XIAP for the efficacy of cisplatin treatment has been studied in a number of studies. Parton and co-workers found no association between XIAP expression and response to chemotherapy in ovarian cancer tissue (Parton et al., 2002). An inverse correlation between XIAP expression in the cancer tissue and pathological response was observed for patients with advanced bladder cancer (Pinho et al., 2009). The correlation, however, was not statistically significant. This study also demonstrated that bladder cancer patients with high levels of XIAP-associated factor 1 protein (XAF1) in the cancer tissue had a better prognosis after cisplatin based chemotherapy (Pinho et al., 2009). XAF1 inhibits the anti-caspase activity of XIAP, therefore antagonizing the anti-apoptotic action (Liston et al., 2001). Most likely, this resulted in increased sensitivity towards cisplatin. Another study investigated the expression of XIAP in bladder tumor specimens of 108 patients and found that XIAP was expressed at significantly higher levels in tumors compared to normal urothelium (Bilim et al., 2003). Unfortunately, it was not investigated, whether XIAP positivity was correlated with clinical response to cisplatin. However, it was suggested that XIAP upregulation might play a role in early TCC carcinogenesis (Bilim et al., 2003).

Altogether, information about expression of factors involved in cisplatin-induced apoptotic cell death pathways and its relation to cisplatin resistance is still emerging (Table 1). More information about the clinical relevance of apoptosis-related factors for the clinical outcome is needed, as this may identify new targets for pharmacological intervention.

5. Strategies for overcoming cisplatin resistance

As cisplatin resistance influences the clinical outcome, strategies are needed to circumvent the resistance phenotype. In a number of preclinical studies, modulators of cisplatin resistance were specifically targeted, and it was investigated whether this would influence cisplatin sensitivity. For example, the glutathione system may be modulated by glutathione depletion or GST blocking agents. Using these approaches, Buyn and co-workers could significantly enhance the cisplatin toxicity in bladder cancer cell lines (Byun et al., 2005). Similarly, inhibition of DNA repair has the potential to enhance the cytotoxicity of anticancer agents, as preclinical studies have confirmed that modulation of repair pathways can enhance the sensitivity to DNA damaging agents (Damia & D'Incalci, 2007, Ding et al., 2006). We found that siRNA-mediated down-regulation of the repair factor ERCC1-XFP decreased the repair of cisplatin-induced ICLs in bladder cancer cells and subsequently resulted in reduced cisplatin resistance (Usanova et al., 2010). In a number of studies, the

effect of down-regulation of anti-apoptotic proteins for cisplatin resistance was studied. Down-regulation of Bcl-2 and Bcl-xL with antisense oligonucleotides enhanced the cisplatin sensitivity in four human bladder cancer cell lines (Bolenz et al., 2007). Antisense oligonucleotides against Bcl-2 were also used by Schaaf and co-workers who also observed an synergistic effect on cisplatin sensitivity (Schaaf et al., 2004). These findings show that reducing anti-apoptotic proteins positively influences cisplatin efficacy in bladder cancer cell lines and imply that targeting these factors may be a new therapeutic strategy for the treatment of bladder cancer.

6. Novel therapeutic strategies for bladder cancer treatment

Gemcitabine (2′,2′-difluorodeoxycytidine) is a deoxycytidine analogue, which can inhibit the ribonucleotide reductase or may be incorporated into DNA as a false base. Both mechanisms result in inhibition of DNA synthesis thereby leading to induction of apoptosis (Mini et al., 2006). Gemcitabine is used either as a single agent or in combination with other chemotherapeutic drugs for the treatment of cancer. For patients with locally advanced and metastatic bladder cancer, combination treatment of cisplatin or carboplatin and gemcitabine is the current standard chemotherapy regimen (von der Maase et al., 2005). Even though drug resistance is a major clinical problem, the resistance phenotype of bladder cancer cells to gemcitabine has not been investigated in great detail. An increase in expression of the anti-apoptotic protein clusterin has been described as a mechanism for acquired gemcitabine resistance in bladder cancer cells (Muramaki et al., 2009). Knock-down of clusterin sensitized gemcitabine-resistant bladder cancer cells indicating clinical significance (Muramaki et al., 2009). Gemcitabine resistance in bladder cancer cells might differ from cisplatin resistance as gemcitabine has been used for the treatment of cisplatin-refractory metastatic bladder cancer (Soga et al., 2010). The beneficial effect of gemcitabine for the treatment of cisplatin-refractory urothelial carcinoma, however, was not observed in the study of Lin and co-workers who reported that gemcitabine and ifosfamide showed insufficient clinical activity in patients with cisplatin-refractory bladder cancer (Lin et al., 2007). More promising approaches to increase the activity of cisplatin plus gemcitabine for treating metastatic bladder cancer have been reported in a number of recent studies. Addition of vitamin D3 increased the antitumor activity of cisplatin plus gemcitabine in bladder cancer cells and enhanced the antitumor activity in a xenograft model (Ma et al., 2010). The antibody Bevacizumab, which is directed against vascular endothelial growth factor (VEGF), has been shown to have a beneficial effect on cisplatin plus gemcitabine in patients with metastatic bladder cancer (Hahn et al., 2011). More clinical trials combining novel agents with cisplatin and gemcitabine, however, are needed to improve the treatment of bladder cancers.

7. Conclusion

Cisplatin-based combination therapy is the standard therapy for the treatment of advanced or metastatic cancer of the bladder. However, the efficacy of cisplatin is limited by intrinsic or acquired resistance to the drug. Mechanisms determining cisplatin resistance include drug transport, detoxification, DNA repair and expression of pro- and anti-apoptotic proteins. The clinical significance of these mechanisms for bladder cancers is not yet fully understood and still evolving. A better understanding about resistance mechanisms in

bladder cancers is essential for developing therapeutic strategies aimed at circumventing cisplatin resistance for improving cancer therapy.

8. References

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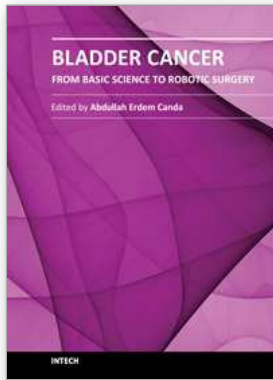
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