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Analysing Molecular Mechanism Related to Therapy-Resistance in *In-vitro* Models of Ovarian Cancer

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Abstract

Ovarian cancer is among the most common cause of cancer death and ranks first in the number of deaths each year in the field of gynaecological malignancies. This is due to its late diagnosis and the development of chemoresistance. Platinum derivatives, including cisplatin and carboplatin in combination with paclitaxel, are the first-line chemotherapeutic agents. Platinum derivatives irreversibly intercalates into the DNA and creates inter- and intra-strand DNA cross-links. During cell division, platinum-DNA-adducts block the replication machinery, inducing DNA damage and apoptosis. Nearly all patients respond to first-line chemotherapy before it comes later to recurrence of the disease. At time of recurrence, tumours are usually more aggressive, form metastasis in secondary tissues and acquire resistance to conventional chemotherapeutics. Drug resistance is a common problem in tumour therapy not only restricted to ovarian cancer. It is characterized by gene mutations, increased DNA repair, reduced drug efficacy and enhanced drug clearance and detoxification. Up to now the complex molecular mechanism of chemoresistance is not well understood. Increasing evidence points towards AKT over-expression and alteration of the PI3K/AKT/mTOR cascade as a central mechanistic reason for this resistance.

Keywords: Ovarian cancer, cisplatin resistance, AKT, PI3K

1. Introduction

There were 14,1 million new cancer cases, 8,2 million cancer deaths and 32,6 million people living with cancer (within 5 years of diagnosis) in 2012 world-wide [1].

Gynaecological tumours are among the most common cause of cancer death and currently causing more than 100,000 deaths per year [2]. Ovarian cancer is an important public health problem because it has the highest tumour-associated mortality of gynaecological malignancies and 239,000 women have been diagnosed with ovarian cancer in 2012 [2]. Furthermore there has been no appreciable improvement in survival for woman with advanced ovarian cancer over the past 40 years. The survival of ovarian cancer is poor and more than 70% of cases are diagnosed at late stage.

In ovarian cancer treatment platinum-based chemotherapy plays a pivotal role as first-line chemotherapy option alone or in combination with taxane [3]. Therefore platinum-resistance is the most crucial problem for treating ovarian cancer. Increasing evidence points towards AKT over-expression and alteration of the PI3K/AKT/mTOR cascade as a mechanistic reason for this resistance.

This chapter provides a short overview of the PI3K/AKT/mTOR-signalling network by summarizing *in-vitro* cell culture based studies that confirm the role of AKT as an important mediator of platinum resistance. The rationale for targeting this pathway in cancer will be discussed with a special focus on tumour immunological aspects also based on *in-vitro* studies. Moreover the PI3K/AKT/mTOR-signalling cascade other general mechanisms of resistance will be shortly addressed. Platinum-resistance can be also caused by differential expression of microRNAs as well as by detoxification of bioactive platinum-complexes by sulphur-containing peptides or proteins, cellular compartmentation, increased DNA repair and alteration in apoptotic signalling pathways [4]. Furthermore diminished drug accumulation caused by reduced uptake or increased efflux of platinum compounds via heavy metal transporter can result in platinum therapy failure [4].

A better understanding of the molecular mechanisms causing cancer therapy-resistance might result in new therapeutic options for patients suffering from tumours.

2. Phosphatidylinositol-3-Kinase (PI3K)/AKT/mTOR-signal transduction pathway

One of the most frequently altered signalling pathways involved in cancer as well as in development of resistance especially in ovarian cancer is the PI3K/AKT/mTOR pathway.

PI3K is a member of the lipid-kinase-family that can phosphorylate the 3'-OH-group of inositolphospholipids as phosphatidylinositol-4,5-bisphosphate (PIP₂) which is converted into the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP₃) [5]. According to different protein structure of the catalytic subunit, PI3Ks are subdivided into three classes.

Class I PI3Ks are the most studied class of PI3K and the most interesting with regard to signalling in tumours. Class I PI3Ks are activated by extracellular signal transduction via receptors with tyrosine-kinase activity or via G-protein coupled receptors (GPCR). In tumour cells growth-factors that bind to the specific receptors activate class 1 PI3Ks and this results in inhibition of autophagy [6].

PI3K activity is associated with cytoskeletal organization, cell division, inhibition of apoptosis and glucose uptake [7-9]. The second messenger PIP_3 in turn activates in the PI3K/AKT-pathway different proteins like AKT (protein kinase B), a serine-threonine kinase [5, 10]. PIP_3 itself is reconverted in PIP_2 via different phosphatases especially PTEN and SHIP [5]. AKT is the key protein in the PI3K/AKT signalling pathway; it binds PIP_3 over the plekstrin-homology-domain (PH-domain) and by this AKT translocates to cell membrane where it interacts with various phospholipids [10]. Cell membrane bound AKT is phosphorylated by phosphoinositide-dependent kinase-1 (PDK1) at threonine 308 and by PDK2 at serine 473 [5, 10, 11]. AKT can also be activated by mTOR2 [5, 12]. Phosphorylated AKT is the active form that modulates and regulates a huge range of proteins involved in diverse cellular processes such as cell cycle regulation, cell proliferation and cell viability [13]. Phosphorylation of AKT can be blocked by the carboxy-terminal modulator-protein (CTMP) and by this preventing the AKT activation as well as further signal transduction [5]. Phosphorylated AKT activates another serine-threonine-kinase, the mammalian target of rapamycin (mTOR) an important regulator for translation, cell growth and cell cycle [14, 15]. Furthermore mTOR has an important role in regulation of autophagy [6, 16, 17].

In general the PI3K/AKT-signal transduction pathway is of pivotal importance for mediating and controlling several cellular processes including cell growth, cell proliferation, survival, motility, adhesion, migration, differentiation, metabolic processes and cell cycle progression in cells [18, 19]. Amplifications, mutations, translocations and deregulation result in aberrant activation of this pathway [5, 20-23]. Furthermore the loss-of-function caused by mutation or deletion of phosphatase and tensin homolog (PTEN) protein results in an increased activity of the PI3K/AKT pathway [6]. The PTEN protein acts as a phosphatase and dephosphorylate PIP_3 , resulting in the biphosphate product PIP_2 . The dephosphorylation is essential as it triggers the inhibition of the AKT signalling pathway [24, 25].

3. Alteration of the PI3K/AKT/mTOR-signal transduction pathway in tumours

Recent studies indicate that numerous components of the PI3K/AKT/mTOR-pathway are deregulated by amplification, mutation and translocation more frequently than any other pathway in cancer patients with resultant activation of this pathway [20].

Both genetic and biochemical data suggest that activation of the PI3K/AKT/mTOR survival pathway contributes to ovarian cancer development and tumourigenesis [15]. Such activation is caused by different mechanisms and one mechanism is somatic alterations in PI3KCA gene that have been found in a substantial fraction of ovarian cancers [26]. PIK3CA amplifications

are present in 40% of ovarian cancers [19]. Furthermore, activation of PI3K/AKT/mTOR signal transduction pathway is caused by mutations in the gene coding for PIK3CA. Another alteration that results in increased activity of the PI3K/AKT/mTOR pathway is PTEN loss-of-function. PTEN loss is observed in about 7% of all ovarian cancer cases and it seems to be more common in type I ovarian tumours [27-32].

For AKT a point-mutation in the PH-domain has been detected in ovarian cancer [33]. This point-mutation results in conformational change of the PH-domain so that AKT can be activated without the presence of PI3K [33].

Deregulation, mutation or over-expression of cell surface receptors can also result in an increased activity of the PI3K/AKT/mTOR signalling pathway in ovarian cancer [34]. Furthermore Ras mutations are found in 20% of low-grade ovarian cancers [35]. Since Ras has been shown to activate both the Ras/Raf/MEK/ERK and the PI3K/AKT/mTOR pathways, mutations of Ras should theoretically activate both pathways simultaneously. Nevertheless so far it has not been evaluated in detail if Ras mutations can result in an increased activity of the PI3K/AKT/mTOR-signalling pathway. Although one study demonstrates that some Ras mutations result in deregulated PI3K and downstream AKT activation [36]. Beside Ras mutations also the over-expression of several other proteins e.g. Rab25 [37], Twist2 [38] or MyD88 [39] seems to enhance activation of AKT. The fact that AKT can be activated by a number of different proteins underlines the key role of AKT signalling under physiological and pathophysiological conditions. As evidence, in human specimens of ovarian cancer AKT was found to be activated in 68% of cases [40].

4. Effects of altered PI3K/AKT/mTOR-signal transduction pathway in tumours

As mentioned before, AKT is an important regulator of various cellular pathways that promote cell survival, cell proliferation, angiogenesis and invasion. Furthermore, the epithelial-mesenchymal-transformation, an important step for tumour metastasis, has been shown to be related to AKT activation [41]. Deregulation of components of the PI3K/AKT-cascade not only contributes to ovarian cancer development and tumourigenesis but also to chemotherapeutic drug and radiation resistance as it was recently shown [4, 5, 18, 42-56]. The sensitivity of cells to radiation and chemotherapeutic drug-induced apoptosis is determined by the balance between cellular survival and apoptosis [5, 12]. Due to the well-known anti-apoptotic role of AKT, an AKT over-expression in cancer cells might be related to increased resistance to radiation and chemotherapy.

Beside the PI3K/AKT/mTOR signalling cascade other general mechanisms of resistance exist. However in this chapter other possibilities of platinum-resistance will be mentioned only shortly.

In general diminished drug accumulation caused by reduced uptake or increased efflux of platinum compounds via heavy metal transporter can result in platinum therapy failure [4].

Furthermore in some resistant cell lines with increased cisplatinum efflux an increased intracellular pH was detected [57]. Intra-cellularly, cisplatinum's chlorides are replaced by neutral hydroxyl or highly reactive positively charged aqua groups, with the pKa for the interconversion between chloro-hydroxy and chloro-aqua species being 6.56 [58]. Hence, if intracellular pH is high, a higher proportion of drug may be represented in the uncharged chloro-hydroxy form, with increased passive efflux of this form.

Another general resistance mechanism is detoxification of bioactive platinum-complexes by sulphur-containing peptides or proteins. Increased glutathione (GSH) level has been shown to cause resistance by binding and inactivating cisplatinum, enhancing DNA repair and reducing cisplatinum-induced oxidative stress [59-62].

Increased DNA repair and reduced apoptotic response are further possible reasons for platinum resistance [4, 63]. Cisplatinum may induce apoptosis through the Fas/Fas ligand signalling complex (with activation of caspase-8, then caspase-3), or by mitochondrial cytochrome-c release [64]. In the presence of ATP and cytochrome-c, apoptotic-protease-activating-factor-1 (Apaf-1) activates caspase-9, with subsequent caspase-3 activation [64]. Cisplatinum may also kill via a caspase-3 independent apoptotic pathway, by a defective apoptotic pathway or by necrosis [64]. Caspase-3, -8 and -9 are important in cisplatinum-induced apoptosis [62]. A cisplatinum-resistant cell showed global down-regulation of caspase and Bax expression, but increased Bcl-2 [65].

Recent reports describe that platinum-resistance can be also caused by differential expression of microRNAs (miRNAs) [66-69]. miRNAs belong to the family of small non-coding RNAs; they are generally 21-25 nucleotides long and play key role in post-transcriptional modulation of gene expression thus representing fine regulators in tumour development and progression as well as response and resistance to anti-tumour agents [70]. miRNA-152 was identified as an autophagy-regulating miRNA down-regulated in cisplatinum-resistant cell lines and also *in-vivo* in ovarian cancer tissues reduced expression has been associated with cisplatinum-resistance. miRNA-152 regulates autophagy by targeting ATG14 the key player in orchestrating autophagy. Thus over-expression of miRNA-152 sensitized cisplatinum-resistant ovarian cancer cells by reducing cisplatinum-induced autophagy, enhancing cisplatinum-induced apoptosis and by inhibition of cell proliferation [69]. Microarray analyses have been used to identify miRNAs involved in cisplatinum-resistance and it was demonstrated that miRNA-21-3p over-expression, the passenger strand of the known oncomiR 5p, increased resistance to cisplatinum in a range of ovarian cell lines [66]. Furthermore a high level of miRNA-490-3p expression was identified as involved in the development of drug resistance against paclitaxel [68]. Another miRNA, miRNA-449a, was found to be down-regulated in cisplatinum-resistant ovarian cancer cells and NOTCH1 was identified as direct target of its modulation [67]. Therefore it is evident that down-regulation as well as over-expression of miRNAs can result in resistance to anti-tumour agents. Recently it was demonstrated that miRNAs involved in platinum-resistance are directly involved in regulation of PTEN, AKT or other downstream molecules of the PI3K/AKT pathway [71-79].

The evidence that members of the PI3K/AKT/mTOR pathway are regulated by miRNAs involved in platinum-resistance increases the importance of the PI3K/AKT/mTOR signalling

cascade as therapeutic target. Therefore inhibition of PI3K/AKT/mTOR signalling in ovarian carcinomas appears a promising target to enhance the efficacy of anticancer agents such as cisplatin and to overcome the resistance of tumour cells against therapy. This hypothesis was tested in different preclinical *in-vitro* studies. Cancer cell lines are frequently used as *in-vitro* tumour models especially for analyzing and studying the effects related to a single gene modification. Nowadays approximately 100 ovarian cancer cell lines are publicly available [80]. Some of these cell lines are known to be platinum resistant e.g. SKOV-3/DDP and Caov-3. Among different ovarian cancer cell lines established there are also the parental A2780 cells and the cisplatin-resistant A2780cis cells [81]. Both cell lines are p53 and K-Ras wild-type and they share the same genetic background. The cisplatin-resistant A2780cis cell line has been developed by chronic exposure of the parental cisplatin-sensitive A2780 cell line to increasing concentrations of the chemotherapeutic agent [81]. These cell lines are excellent models for analyzing the molecular basis for cisplatin resistance in ovarian cancer [47-49, 82-85]. According to these studies AKT over-expression in ovarian cancer is strongly related to platinum resistance in this specific tumour [37, 47, 86]. It was shown that high AKT protein expression is strongly associated to cisplatin-resistant A2780cis cell line compared to the parental A2780 cell lines [47, 48]. The platinum resistance in A2780cis cell line could be overcome by AKT down-regulation via siRNA [47]. This was demonstrated in several functional *in-vitro* assays, e.g. clonogenicity assays and irradiation assays (Figure 1), as by determination of the apoptosis rate. Furthermore the cytotoxicity of cisplatin was addressed in proliferation assays. Stable increase of AKT amount in the cell lines results in an increased IC₅₀ value for cisplatin whereas a stable decrease of AKT results in an increased accessibility for cisplatin treatment [47].

However in the same isogenic model it was shown that AKT-over-expression was able to transform platinum-sensitive A2780 cells into platinum-resistant. On the contrary, platinum-resistance of A2780cis cells could be reversed by down-regulation of AKT [47]. FACS analysis demonstrated also that cisplatin induces cell cycle arrest predominantly in the S and the G2/M phase but also in the G1 phase regardless of the AKT-expression status (Figure 2). However, required doses of cisplatin to induce cell cycle arrest were progressively higher in cell lines with AKT over-expressed [47, 87].

As already mentioned above the sensitivity of cells to radiation and drug-induced apoptosis is determined by the balanced expression between pro-apoptotic and anti-apoptotic proteins [5, 12]. Therefore the effect of the PI3K/AKT cascade on pro-apoptotic protein like BAD, a known substrate of AKT, has been studied in both cisplatin-resistant Caov-3 and sensitive A2780 human ovarian cancer cells [88]. Treatment of Caov-3 and A2780 cells with cisplatin was able to stimulate the activation of AKT, whereas the PI3K inhibitor wortmannin blocked the cisplatin-induced AKT activation. Cisplatin treatment was capable to activate phosphorylation of BAD at Ser-112 and Ser-136 sites in Caov-3 and A2780 cells. While phosphorylation of BAD at Ser-136 was blocked by treatment with wortmannin, its phosphorylation at Ser-112 was blocked by a MAP/ERK kinase inhibitor PD98059 [89]. Transient exogenous expression of a dominant-negative AKT in both Caov-3 and A2780 cells decreased cell viability after treatment with cisplatin. In contrast, no sensitization to cisplatin was

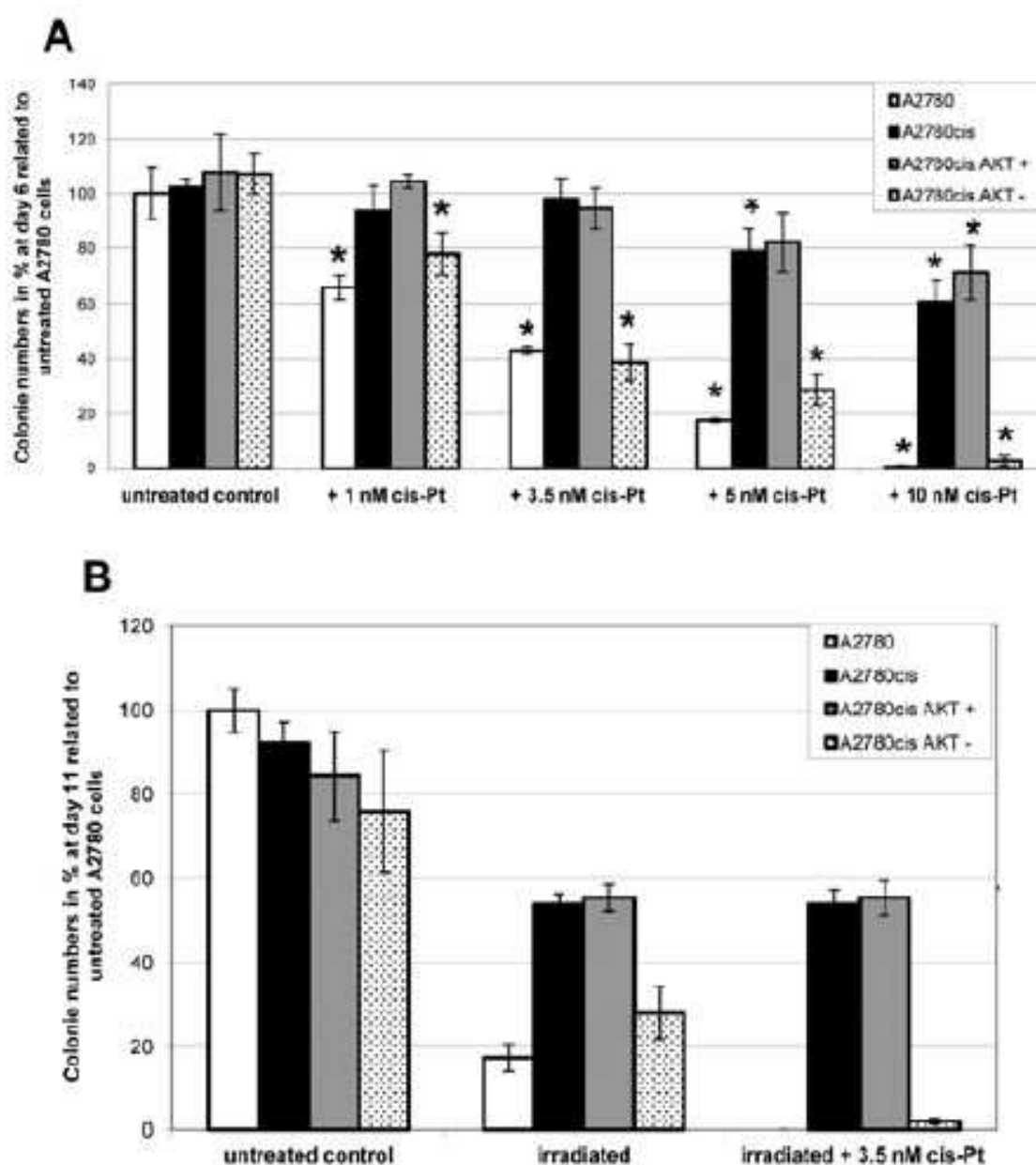


Figure 1. Clonogenicity-Assays. (A) Cells were treated with different concentrations of cisplatin (cis-Pt) for 6 days and (B) cells were first irradiated with 2.5 Gray and then treated with 3.5 nM cisplatin (cis-Pt) for 11 days. Cells were stained and fixed with crystal violet. The formed cell colonies were counted. The figure shows the colony numbers in relation to the colonies formed by untreated A2780 (set to 100%). Three independent experiments were performed, and each experiment was carried out in triplicate. Statistically significant difference ($p < 0.05$) between a sample and the relevant control is indicated by *. All data were previously published in "Oncology Reports" [47] and is reprinted by permission of Spandidos Publications ©2012.

observed in cells expressing wild-type AKT. These findings suggested that cisplatin-induced DNA damage causes phosphorylation of BAD via an extracellular signal-regulated protein kinase (ERK) cascade and via a PI3K/AKT/mTOR cascade. Inhibition of both cascades enhance the sensitivity of ovarian cancer cells to cisplatin thus providing further evidence that AKT-pathway is involved in cisplatin resistance in ovarian cancers [88]. Additional

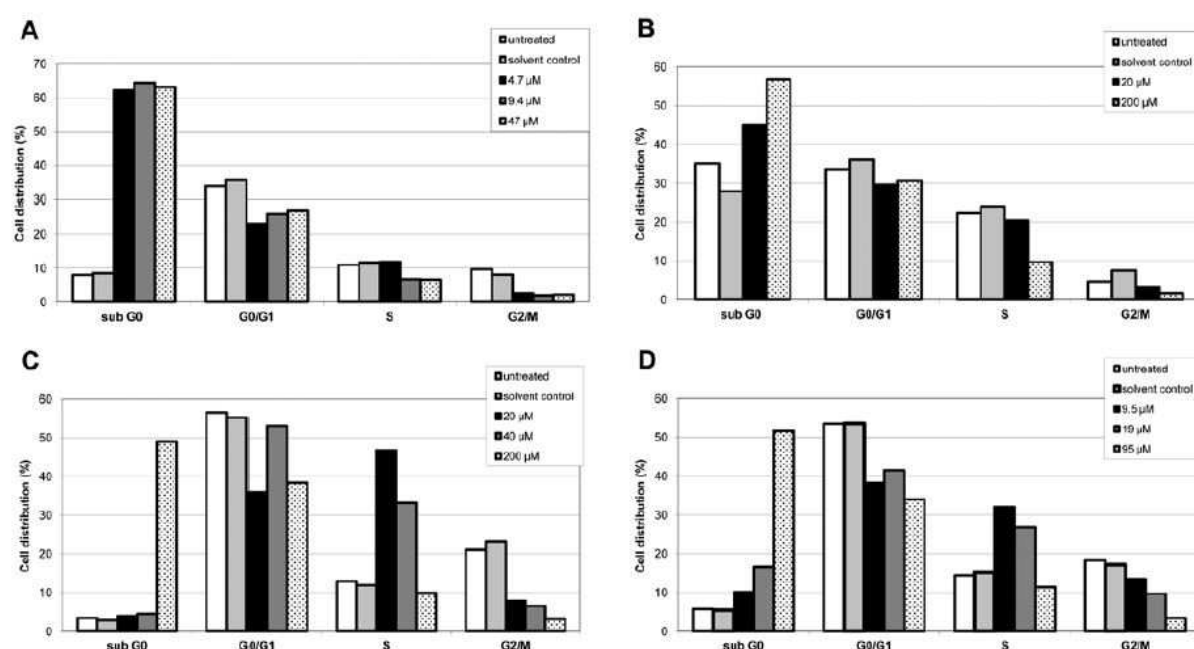


Figure 2. Effect of cisplatin (cis-Pt) on the cell cycle distribution. A2780 (A), A2780cis (B), A2780cis AKT+ (C) and A2780cis AKT- (D) cells were treated with different concentrations of cisplatin (cis-Pt) for 24 h, fixed, permeabilized, stained with propidium iodide and analysed by flow cytometry. The figure shows the distribution of the cells to the different phases of cell cycle (%). All data were previously published in "Oncology Reports" [47] and is reprinted by permission of Spandidos Publications ©2012.

results suggest that AKT confers platinum-resistance in part by modulating the direction of p53 on the caspase-dependent mitochondrial death pathway [90]. Thus in ovarian cancers while p53 is a determinant for platinum sensitivity AKT contributes to chemoresistance in part by attenuating p53-mediated PUMA upregulation and phosphorylation of p53 [91]. Recent results suggest that in platinum sensitive ovarian cancer cells cisplatin-induced apoptosis can also proceed to a lesser extent via a caspase-independent mechanism involving apoptosis inducing factor (AIF) and that AKT activation additionally confers resistance to cisplatin-induced apoptosis by blocking this pathway [90].

A recent work evaluated the anti-tumour efficacy of the AKT inhibitor perifosine in platinum-sensitive and -resistant human ovarian cancer cells [45, 92]. In different ovarian cancer cell lines and *in-vivo* experiments it has been possible to show that cells with higher levels of phospho-AKT are more sensitive to treatment with AKT-inhibitor perifosine. Furthermore, coincubation with perifosine sensitized A2780cis cells to treatment with cisplatin. AKT-inhibitor perifosine has already been tested in phase II studies in patients with breast, prostate, pancreatic, head and neck, colorectal cancer, malignant melanoma, multiple myeloma, and soft tissue sarcoma [93-99]. A recent phase I study with perifosine combined with radiotherapy performed in patients with advanced solid tumours has shown preliminary evidence of anti-cancer activity, including complete responses [100]. Thus, perifosine seems to be an attractive compound for further clinical studies in particular phenotypes tumour like platinum-resistant ovarian cancers.

New attractive therapeutic targets are presented by the PI3K/AKT/mTOR-pathway activating cell surface receptors like vascular endothelial growth factor (VEGF) receptors [101]. VEGF is a key activator of angiogenesis, a physiological multi-step process that includes endothelial cell growth and movement [102]. It plays important roles in wound healing and endothelial-cell-mediated degradation of the extracellular matrix, as well as the transition of benign tissue into solid tumours [102-104]. Recent studies have suggested that the PI3K/AKT signalling cascade may be implicated in tumour angiogenesis [105-107]. In clinical trial studies, high VEGF levels have been negatively correlated with survival of patients. Ovarian cancer cells over-expressing VEGF own a metastatic advantage over those VEGF low expressing [108, 109] and even more higher levels of serum VEGF are found in patients with metastasis if compared to metastasis-free patients [110]. Moreover, down-modulation of VEGF has been shown to inhibit tumour growth and to suppress tumour invasion and metastasis. These findings have laid the basis for the clinical evaluation of agents targeting VEGF signaling pathway in patients with ovarian cancer [101]. Bevacizumab (Avastin) has been the first and most studied anti-VEGF agent in clinical evaluation for ovarian cancer [111]. Bevacizumab showed additive or synergistic effects in combinational therapy with paclitaxel and marked reduction of tumour growth and ascites formation [112]. Using a murine ovarian cancer model a significant antitumour activity of Bevacizumab as a single agent or in combination with cisplatin was demonstrated [113]. In the meantime Bevacizumab was approved as a treatment in combination with paclitaxel, topotecan, or pegylated liposomal doxorubicin chemotherapy for women with recurrent ovarian cancer that are resistant to platinum-based chemotherapy [114-117].

Furthermore, other agents targeting VEGF receptors have also been evaluated for the use in treatment of ovarian cancer as Ramucirumab, a fully humanized monoclonal antibody, that specifically block VEGFR-2 resulting in reduced tumour growth, increased apoptosis and decreased tumour microvessel proliferation and density [118]. Following a phase I evaluation [119], it is currently being assessed in a phase II trial as monotherapy in patients with platinum-refractory persistent or recurrent epithelial ovarian cancer.

5. Role of AKT expression level in tumour cells in regard to NK killing

Another important aspect in cancer development and progression is the role of the immune system. Since survival is strongly influenced by immunological parameters, immunotherapeutic strategies appear promising and for this reason during the last years the interest in tumour immunology has constantly increased. A necessary prerequisite for immunotherapy in patients is a better understanding of the interaction between ovarian tumour cells and cells of the immune system especially with natural-killer (NK)-cells. NK-cells are a critical component of the innate immune response against infectious pathogens and malignant transformation [120, 121]. NK-cells mediate this activity through the elaboration of various cytokines as well as through direct cytolytic activity. However, unlike adaptive immune cells, which utilize specific clonal recognition receptors, NK-cell activation depends on a complex balance between activating and inhibitory signals [122, 123]. Nevertheless, NK-cells play an important

role in immune surveillance and coordinating responses of other immune cells. Most tumour cells express surface molecules that can be recognized by activating receptors on NK-cells [124]. The expression of these receptors make such cells susceptible to endogenous NK-cells, but malignant cells have developed mechanisms to evade these mechanisms of innate immune surveillance [125-127]. In patients with cancer, it is presumed that tumour cells have developed mechanisms to suppress NK-cell activation and resist lysis by endogenous NK-cells, but the molecular basis for target resistance is not well understood. Recent studies have suggested that AKT can regulate the development and functions of innate immune cells [128] thus providing evidence that AKT plays also an important role in immune modulation. However in this chapter will be addressed only the role of activated AKT in tumour cells in regard to NK-cells.

Dysregulated cytokine release can either lead to or be associated with a failure in cell-cell recognition thus allowing cancer cells to evade the killing system. The PI3K/AKT/mTOR pathway regulates multiple cellular processes which underlie immune responses against pathogens or malignant cells [129, 130]. Conversely, there is accumulating evidence that the PI3K/AKT/mTOR pathway is involved in the development of several malignant traits of cancer cells as well as their escape from immunity [131]. In some studies the interactions between cancer cells and natural-killer (NK)-cells have been enlightened [48, 82, 132-134]. Modified FATAL assay was used for determining the killing efficiency of NK-cells in regard to ovarian cancer cell models *in-vitro* (Figure 3).

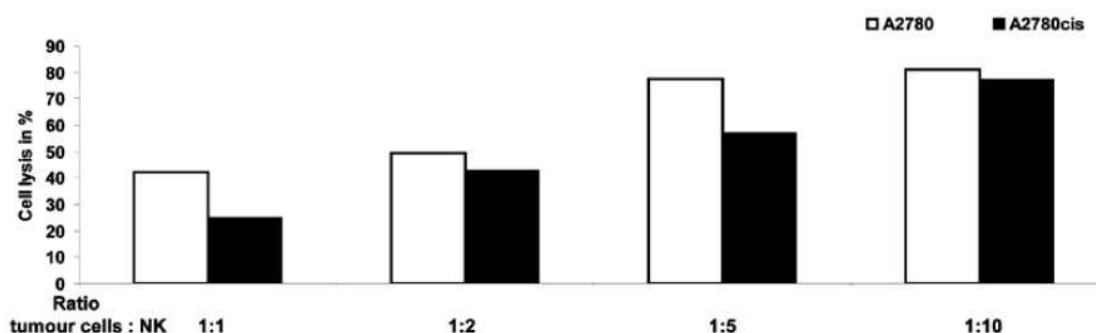


Figure 3. Lytic activity of polyclonal natural-killer (NK)-cells. A2780 and A2780cis cells (10^5 cells/well), respectively, were used as targets in a modified 5 h FATAL assay using various tumour cell : NK-cell ratios. Target cell lysis was determined by flow cytometric analysis. The percentage of tumour cell lysis was determined in relation to a control containing tumour cells with medium alone. A representative of three independent experiments is shown. All data were previously published in "International Journal of Oncology" [48] and is reprinted by permission of Spandidos Publications ©2013.

In this model parental A2780 cells and the cisplatin resistant A2780cis human ovarian cancer cells have been used. The efficiency of NK-cell mediated cell lysis differs between A2780 cells and the cisplatin-resistant A2780cis cells. The A2780cis cells are less accessible for NK-cell mediated killing [48, 82] and this findings are in agreement with a report by Bellucci *et al.* [135]. Using a lentiviral shRNA library targeting >1,000 human genes they identified 83

genes that promote target cell resistance to human NK-cell-mediated killing [135]. Many of the genes discovered by this screening belong to common signalling pathways including multiple members of the AKT/PI3K/mTOR pathway as PIK3CA and PIK3CB [135]. The comparison of the cancer cell lines A2780 and A2780cis revealed that the differences observed with regard to NK-cell mediated killing rely mainly on two mechanisms. Firstly, the observed increased expression of anti-apoptotic genes (especially *ciap-1* and *-2*) in A2780cis cells compared to A2780 cells is able to confer resistance to A2780cis cells to apoptosis. Second, the CD112 ligand for NK-cell receptor DNAM-1 was expressed at a lower level in A2780cis cells though ligands for the NK-cell receptor NKG2D, e.g. MICA/B, were more strongly expressed in the platinum-resistant cells than in the parental A2780 cells [48]. Moreover A2780cis cells expressed lower levels of TIMP-3, the inhibitor of MICA/B shedding, whereas specific proteases for shedding were also found expressed and this resulted in a net increase of soluble MICA/B in A2780cis cell lines [48]. It is well known that cleaved MICA/B protects cells against NK mediated cell killing [48, 136, 137]. Therefore, it is reasonable to speculate that the increased amount of soluble MICA/B is responsible for the lower killing rate of platinum-resistant A2780cis cells compared to their parental A2780 cells [48]. It was previously well demonstrated that PI3K/AKT/mTOR pathway is involved in inducing MICA/B expression in breast cancer cells [138]. Overall these findings indicate a more general effect of induced PI3K/AKT/mTOR signal transduction pathway. As well as in breast, in ovarian cancer cells with an increase of phosphorylated AKT-activated, PI3K/AKT/mTOR pathway higher MICA/B expression has been also detected [48]. Recently it has been demonstrated that treatment of tumour cells with JAK inhibitors increased their susceptibility to NK-cell mediated killing [135]. The authors suggested that common signalling pathways can regulate susceptibility of human tumour cells to the surveillance and killing ability of the immunologic effector cells and that small molecules inhibitors of JAK may have promising immunologic effects *in-vivo* [135]. Whether or not inhibition of PI3K/AKT/mTOR pathway might render the platinum-resistant A2780cis cells accessible for NK-cell mediated killing must be evaluated in further studies. Only the few first steps towards the characterization of the molecular basis of resistance mechanisms in ovarian cancer with different AKT expression levels in the context of NK-cell mediated killing are being explored [48, 82].

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