We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

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

Jens C. Hahne, Arnd Honig, Jörg B. Engel, Andrea Lampis and Nicola Valeri

Additional information is available at the end of the chapter

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 derivates, including cisplatinum and carboplatin in combination with paclitaxel, are the first-line chemotherapeutic agents. Platinum derivates 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, cisplatinum 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 (PIP_2) which is converted into the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3) [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]. Further-more 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 cyto-chrome-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 cispla-tinum-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 cisplatinum 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 cisplatinum-resistant A2780cis cells [81]. Both cell lines are p53 and K-Ras wild-type and they share the same genetic background. The cisplatinum-resistant A2780cis cell line has been developed by chronic exposure of the parental cisplatinum-sensitive A2780 cell line to increasing concentrations of the chemotherapeutic agent [81]. These cell lines are excellent models for analyzing the molecular basis for cisplatinum 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 cisplatinum-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 cisplatinum was addressed in proliferation assays. Stable increase of AKT amount in the cell lines results in an increased IC_{50} value for cisplatinum whereas a stable decrease of AKT results in an increased accessibility for cisplatinum 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 cisplatinum 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 cisplatinum 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 cisplatinum-resistant Caov-3 and sensitive A2780 human ovarian cancer cells [88]. Treatment of Caov-3 and A2780 cells with cisplatinum was able to stimulate the activation of AKT, whereas the PI3K inhibitor wortmannin blocked the cisplatinum-induced AKT activation. Cisplatinum 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 cisplatinum. In contrast, no sensitization to cisplatinum was
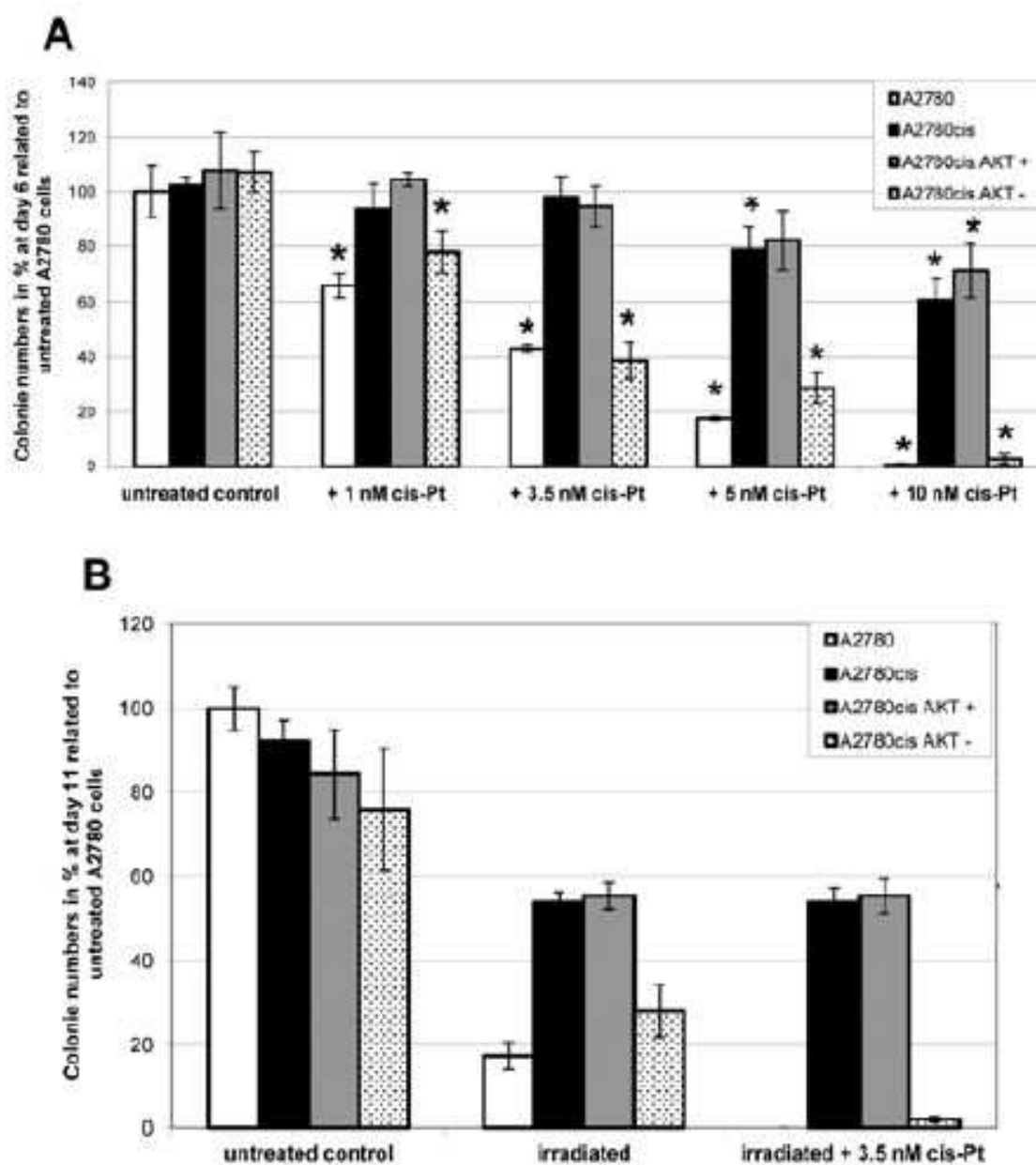
Figure 1. Clonogenicity-Assays. (A) Cells were treated with different concentrations of cisplatin (cis-Pt) for 6 days and (B) cells were first irradiated with 2.5 Gray and then treated with 3.5 nM cisplatin (cis-Pt) for 11 days. Cells were stained and fixed with crystal violet. The formed cell colonies 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 cisplatinum-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 cisplatinum thus providing further evidence that AKT-pathway is involved in cisplatinum resistance in ovarian cancers [88]. Additional
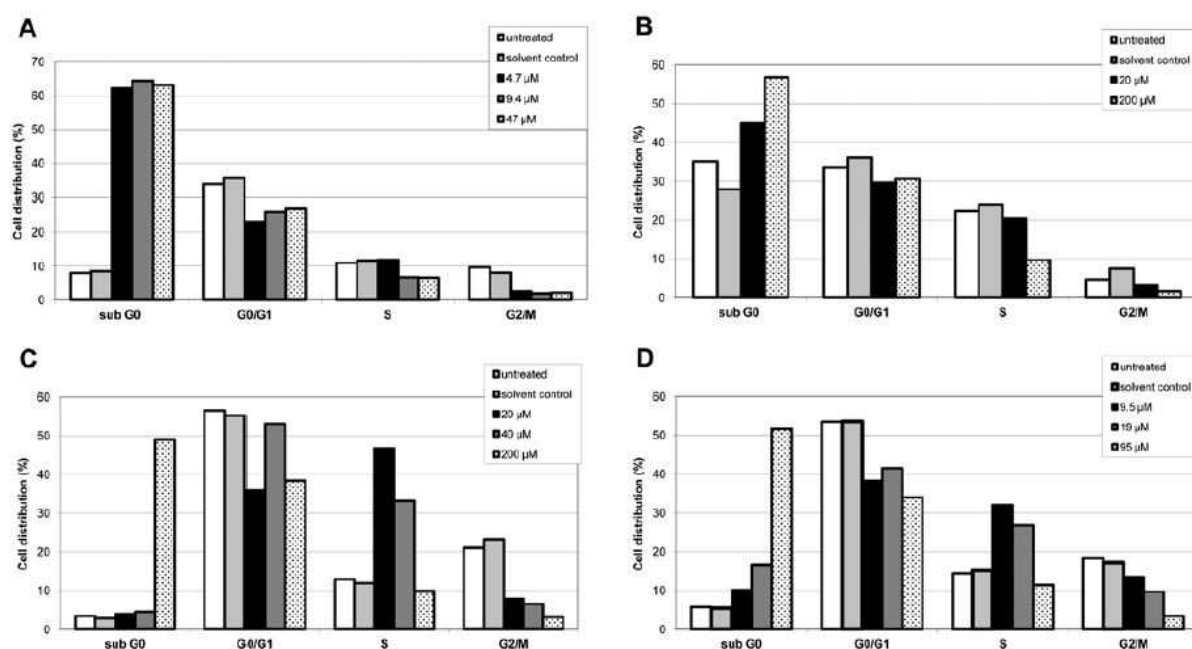
Figure 2. Effect of cisplatin (cis-Pt) on the cell cycle distribution. A2780 (A), A2780cis (B), A2780cis AKT+ (C) and A2780cis AKT- (D) cells were treated with different concentrations of cisplatin (cis-Pt) for 24 h, fixed, permeabilzed, stained with propidium iodide and analysed by flow cytometry. The 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 cisplatinum-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 cisplatinum-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 cisplatinum. 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 cisplatinum 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).
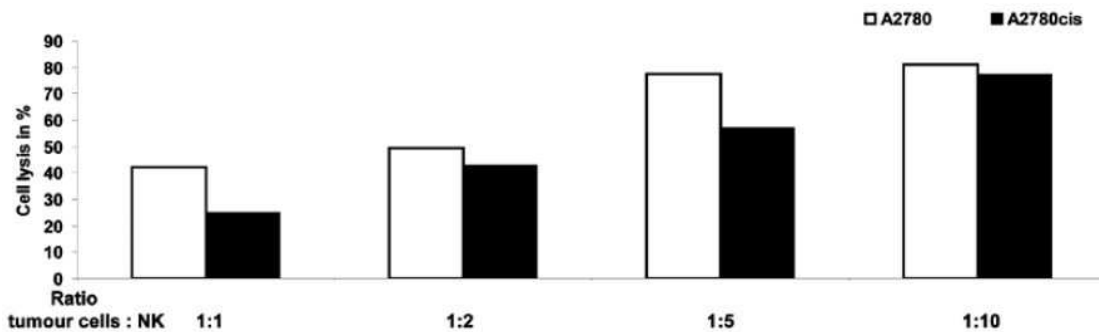

Figure 3. Lytic activity of polyclonal natural-killer (NK)-cells. A2780 and A2780cis cells (10^5 cells/well), respectively, were used as targets in a modified 5 h FATAL assay using various tumour cell : NK-cell ratios. Target cell lysis was determined by flow cytometric analysis. The percentage of tumour 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 cisplatinum resistant A2780cis human ovarian cancer cells have been used. The efficiency of NK-cell mediated cell lysis differs between A2780 cells and the cisplatinum-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].

Author details

Jens C. Hahne[1*], Arnd Honig[2], Jörg B. Engel[3], Andrea Lampis[1] and Nicola Valeri[1]

*Address all correspondence to: jens.hahne@icr.ac.uk

1 Centre for Molecular Pathology, The Institute of Cancer Research, London, UK

2 Gynaecological Hospital, Catholic Clinical Centre Mainz, Germany

3 Department of Obstetrics and Gynaecology, Medical University Gießen, Germany

References

[1]   Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5): E359-386.10.1002/ijc.29210

[2]   Jemal A, Siegel R, Xu J, & Ward E Cancer statistics, 2010. CA Cancer J Clin. 2010;60(5): 277-300.10.3322/caac.20073

[3]   Metzger-Filho O, Moulin C, & D'Hondt V First-line systemic treatment of ovarian cancer: a critical review of available evidence and expectations for future directions. Curr Opin Oncol. 2010;22(5): 513-520.10.1097/CCO.0b013e32833ae99c

[4]   Stewart DJ Mechanisms of resistance to cisplatin and carboplatin. Crit Rev Oncol Hematol. 2007;63(1): 12-31.10.1016/j.critrevonc.2007.02.001

[5]   Vivanco I & Sawyers CL The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002;2(7): 489-501.10.1038/nrc839

[6]   Kondo Y, Kanzawa T, Sawaya R, & Kondo S The role of autophagy in cancer development and response to therapy. Nat Rev Cancer. 2005;5(9): 726-734.10.1038/nrc1692

[7]   Roche S, Koegl M, & Courtneidge SA The phosphatidylinositol 3-kinase alpha is required for DNA synthesis induced by some, but not all, growth factors. Proc Natl Acad Sci U S A. 1994;91(19): 9185-9189

[8]   Wennstrom S, Hawkins P, Cooke F, Hara K, Yonezawa K, et al. Activation of phosphoinositide 3-kinase is required for PDGF-stimulated membrane ruffling. Curr Biol. 1994;4(5): 385-393

[9]   Yao R & Cooper GM Growth factor-dependent survival of rodent fibroblasts requires phosphatidylinositol 3-kinase but is independent of pp70S6K activity. Oncogene. 1996;13(2): 343-351

[10] Toker A & Yoeli-Lerner M Akt signaling and cancer: surviving but not moving on. Cancer Res. 2006;66(8): 3963-3966.10.1158/0008-5472.CAN-06-0743

[11] Kunstle G, Laine J, Pierron G, Kagami Si S, Nakajima H, et al. Identification of Akt association and oligomerization domains of the Akt kinase coactivator TCL1. Mol Cell Biol. 2002;22(5): 1513-1525

[12] Engelman JA Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009;9(8): 550-562.10.1038/nrc2664

[13] Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C, et al. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev. 2004;30(2): 193-204.10.1016/j.ctrv.2003.07.007

[14] Shaw RJ & Cantley LC Ras, PI(3)K and mTOR signalling controls tumour cell growth. Nature. 2006;441(7092): 424-430.10.1038/nature04869

[15] Diaz-Padilla I, Duran I, Clarke BA, & Oza AM Biologic rationale and clinical activity of mTOR inhibitors in gynecological cancer. Cancer Treat Rev. 2012;38(6): 767-775.10.1016/j.ctrv.2012.02.001

[16] Jung CH, Ro SH, Cao J, Otto NM, & Kim DH mTOR regulation of autophagy. FEBS Lett. 2010;584(7): 1287-1295.10.1016/j.febslet.2010.01.017

[17] Wang CW & Klionsky DJ The molecular mechanism of autophagy. Mol Med. 2003;9(3-4): 65-76

[18] Santiskulvong C, Konecny GE, Fekete M, Chen KY, Karam A, et al. Dual targeting of phosphoinositide 3-kinase and mammalian target of rapamycin using NVP-BEZ235 as a novel therapeutic approach in human ovarian carcinoma. Clin Cancer Res. 2011;17(8): 2373-2384.10.1158/1078-0432.CCR-10-2289

[19] Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, et al. PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet. 1999;21(1): 99-102.10.1038/5042

[20] Hennessy BT, Smith DL, Ram PT, Lu Y, & Mills GB Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov. 2005;4(12): 988-1004.10.1038/nrd1902

[21] Bellacosa A, Kumar CC, Di Cristofano A, & Testa JR Activation of AKT kinases in cancer: implications for therapeutic targeting. Adv Cancer Res. 2005;94: 29-86.10.1016/S0065-230X(05)94002-5

[22] Manning BD & Cantley LC AKT/PKB signaling: navigating downstream. Cell. 2007;129(7): 1261-1274.10.1016/j.cell.2007.06.009

[23] Engelman JA, Luo J, & Cantley LC The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet. 2006;7(8): 606-619.10.1038/nrg1879

[24] Kong D, Suzuki A, Zou TT, Sakurada A, Kemp LW, et al. PTEN1 is frequently mutated in primary endometrial carcinomas. Nat Genet. 1997;17(2): 143-144.10.1038/ng1097-143

[25] Minaguchi T, Yoshikawa H, Oda K, Ishino T, Yasugi T, et al. PTEN mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. Clin Cancer Res. 2001;7(9): 2636-2642

[26] Samuels Y & Velculescu VE Oncogenic mutations of PIK3CA in human cancers. Cell Cycle. 2004;3(10): 1221-1224

[27] Kurman RJ & Shih Ie M Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. Hum Pathol. 2011;42(7): 918-931.10.1016/j.humpath.2011.03.003

[28] Vazquez F & Sellers WR The PTEN tumor suppressor protein: an antagonist of phosphoinositide 3-kinase signaling. Biochim Biophys Acta. 2000;1470(1): M21-35

[29] Wu X, Senechal K, Neshat MS, Whang YE, & Sawyers CL The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase/Akt pathway. Proc Natl Acad Sci U S A. 1998;95(26): 15587-15591

[30] Leslie NR, Gray A, Pass I, Orchiston EA, & Downes CP Analysis of the cellular functions of PTEN using catalytic domain and C-terminal mutations: differential effects of C-terminal deletion on signalling pathways downstream of phosphoinositide 3-kinase. Biochem J. 2000;346 Pt 3: 827-833

[31] Dahia PL, Aguiar RC, Alberta J, Kum JB, Caron S, et al. PTEN is inversely correlated with the cell survival factor Akt/PKB and is inactivated via multiple mechanismsin haematological malignancies. Hum Mol Genet. 1999;8(2): 185-193

[32] Hashiguchi Y, Tsuda H, Inoue T, Berkowitz RS, & Mok SC PTEN expression in clear cell adenocarcinoma of the ovary. Gynecol Oncol. 2006;101(1): 71-75.10.1016/j.ygyno.2005.09.047

[33] Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007;448(7152): 439-444.10.1038/nature05933

[34] McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Montalto G, et al. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. Oncotarget. 2012;3(9): 954-987

[35] Ulku AS, Schafer R, & Der CJ Essential role of Raf in Ras transformation and deregulation of matrix metalloproteinase expression in ovarian epithelial cells. Mol Cancer Res. 2003;1(14): 1077-1088

[36] Taylor V, Wong M, Brandts C, Reilly L, Dean NM, et al. 5' phospholipid phosphatase SHIP-2 causes protein kinase B inactivation and cell cycle arrest in glioblastoma cells. Mol Cell Biol. 2000;20(18): 6860-6871

[37] Fan Y, Wang L, Han X, Liu X, & Ma H Rab25 is responsible for phosphoinositide 3-kinase/AKTmediated cisplatin resistance in human epithelial ovarian cancer cells. Mol Med Rep. 2015;11(3): 2173-2178.10.3892/mmr.2014.2963

[38] Wang T, Li Y, Tuerhanjiang A, Wang W, Wu Z, et al. Twist2 contributes to cisplatin-resistance of ovarian cancer through the AKT/GSK-3beta signaling pathway. Oncol Lett. 2014;7(4): 1102-1108.10.3892/ol.2014.1816

[39] Wang Y, Tu Q, Yan W, Xiao D, Zeng Z, et al. CXC195 suppresses proliferation and inflammatory response in LPS-induced human hepatocellular carcinoma cells via regulating TLR4-MyD88-TAK1-mediated NF-kappaB and MAPK pathway. Biochem Biophys Res Commun. 2015;456(1): 373-379.10.1016/j.bbrc.2014.11.090

[40] Levine DA, Bogomolniy F, Yee CJ, Lash A, Barakat RR, et al. Frequent mutation of the PIK3CA gene in ovarian and breast cancers. Clin Cancer Res. 2005;11(8): 2875-2878.10.1158/1078-0432.CCR-04-2142

[41] Chen R, Yang Q, & Lee JD BMK1 kinase suppresses epithelial-mesenchymal transition through the Akt/GSK3beta signaling pathway. Cancer Res. 2012;72(6): 1579-1587.10.1158/0008-5472.CAN-11-2055

[42] Lee S, Choi EJ, Jin C, & Kim DH Activation of PI3K/Akt pathway by PTEN reduction and PIK3CA mRNA amplification contributes to cisplatin resistance in an ovarian cancer cell line. Gynecol Oncol. 2005;97(1): 26-34.10.1016/j.ygyno.2004.11.051

[43] Gagnon V, Mathieu I, Sexton E, Leblanc K, & Asselin E AKT involvement in cisplatin chemoresistance of human uterine cancer cells. Gynecol Oncol. 2004;94(3): 785-795.10.1016/j.ygyno.2004.06.023

[44] Mondesire WH, Jian W, Zhang H, Ensor J, Hung MC, et al. Targeting mammalian target of rapamycin synergistically enhances chemotherapy-induced cytotoxicity in breast cancer cells. Clin Cancer Res. 2004;10(20): 7031-7042.10.1158/1078-0432.CCR-04-0361

[45] Engel JB, Schonhals T, Hausler S, Krockenberger M, Schmidt M, et al. Induction of programmed cell death by inhibition of AKT with the alkylphosphocholine perifosine in in vitro models of platinum sensitive and resistant ovarian cancers. Arch Gynecol Obstet. 2011;283(3): 603-610.10.1007/s00404-010-1457-6

[46] Benedetti V, Perego P, Luca Beretta G, Corna E, Tinelli S, et al. Modulation of survival pathways in ovarian carcinoma cell lines resistant to platinum compounds. Mol Cancer Ther. 2008;7(3): 679-687.10.1158/1535-7163.MCT-07-0450

[47] Hahne JC, Honig A, Meyer SR, Gambaryan S, Walter U, et al. Downregulation of AKT reverses platinum resistance of human ovarian cancers in vitro. Oncol Rep. 2012;28(6): 2023-2028.10.3892/or.2012.2041

[48] Hahne JC, Meyer SR, Gambaryan S, Walter U, Dietl J, et al. Immune escape of AKT overexpressing ovarian cancer cells. Int J Oncol. 2013;42(5): 1630-1635.10.3892/ijo. 2013.1846

[49] Honig A, Hahne JC, Meyer S, Kranke P, Hausler S, et al. PI3K inhibitor D-116883 is effective in in vitro models of ovarian cancer. Anticancer Res. 2012;32(5): 2035-2041

[50] Eckstein N Platinum resistance in breast and ovarian cancer cell lines. J Exp Clin Cancer Res. 2011;30: 91.10.1186/1756-9966-30-91

[51] Cheng JQ, Lindsley CW, Cheng GZ, Yang H, & Nicosia SV The Akt/PKB pathway: molecular target for cancer drug discovery. Oncogene. 2005;24(50): 7482-7492.10.1038/sj.onc.1209088

[52] Hovelmann S, Beckers TL, & Schmidt M Molecular alterations in apoptotic pathways after PKB/Akt-mediated chemoresistance in NCI H460 cells. Br J Cancer. 2004;90(12): 2370-2377.10.1038/sj.bjc.6601876

[53] Westfall SD & Skinner MK Inhibition of phosphatidylinositol 3-kinase sensitizes ovarian cancer cells to carboplatin and allows adjunct chemotherapy treatment. Mol Cancer Ther. 2005;4(11): 1764-1771.10.1158/1535-7163.MCT-05-0192

[54] Testa JR & Bellacosa A AKT plays a central role in tumorigenesis. Proc Natl Acad Sci U S A. 2001;98(20): 10983-10985.10.1073/pnas.211430998

[55] Nicholson KM & Anderson NG The protein kinase B/Akt signalling pathway in human malignancy. Cell Signal. 2002;14(5): 381-395

[56] Dobbin ZC & Landen CN The Importance of the PI3K/AKT/MTOR Pathway in the Progression of Ovarian Cancer. Int J Mol Sci. 2013;14(4): 8213-8227.10.3390/ijms14048213

[57] Chau Q & Stewart DJ Cisplatin efflux, binding and intracellular pH in the HTB56 human lung adenocarcinoma cell line and the E-8/0.7 cisplatin-resistant variant. Cancer Chemother Pharmacol. 1999;44(3): 193-202.10.1007/s002800050967

[58] Andersson A, Hedenmalm H, Elfsson B, & Ehrsson H Determination of the acid dissociation constant for cis-diammineaquachloroplatinum(II) ion. A hydrolysis product of cisplatin. J Pharm Sci. 1994;83(6): 859-862

[59] Perego P, Romanelli S, Carenini N, Magnani I, Leone R, et al. Ovarian cancer cisplatin-resistant cell lines: multiple changes including collateral sensitivity to Taxol. Ann Oncol. 1998;9(4): 423-430

[60] Fokkema E, Groen HJ, Helder MN, de Vries EG, & Meijer C JM216-, JM118-, and cisplatin-induced cytotoxicity in relation to platinum-DNA adduct formation, glutathione levels and p53 status in human tumour cell lines with different sensitivities to cisplatin. Biochem Pharmacol. 2002;63(11): 1989-1996

[61] Byun SS, Kim SW, Choi H, Lee C, & Lee E Augmentation of cisplatin sensitivity in cisplatin-resistant human bladder cancer cells by modulating glutathione concentrations and glutathione-related enzyme activities. BJU Int. 2005;95(7): 1086-1090.10.1111/j.1464-410X.2005.05472.x

[62] Siddik ZH Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene. 2003;22(47): 7265-7279.10.1038/sj.onc.1206933

[63] Richardson A & Kaye SB Drug resistance in ovarian cancer: the emerging importance of gene transcription and spatio-temporal regulation of resistance. Drug Resist Updat. 2005;8(5): 311-321.10.1016/j.drup.2005.09.001

[64] Gonzalez VM, Fuertes MA, Alonso C, & Perez JM Is cisplatin-induced cell death always produced by apoptosis? Mol Pharmacol. 2001;59(4): 657-663

[65] Achanzar WE, Webber MM, & Waalkes MP Altered apoptotic gene expression and acquired apoptotic resistance in cadmium-transformed human prostate epithelial cells. Prostate. 2002;52(3): 236-244.10.1002/pros.10106

[66] Pink RC, Samuel P, Massa D, Caley DP, Brooks SA, et al. The passenger strand, miR-21-3p, plays a role in mediating cisplatin resistance in ovarian cancer cells. Gynecol Oncol. 2015.10.1016/j.ygyno.2014.12.042

[67] Zhou Y, Chen Q, Qin R, Zhang K, & Li H MicroRNA-449a reduces cell survival and enhances cisplatin-induced cytotoxicity via downregulation of NOTCH1 in ovarian cancer cells. Tumour Biol. 2014;35(12): 12369-12378.10.1007/s13277-014-2551-3

[68] Chen S, Chen X, Xiu YL, Sun KX, Zong ZH, et al. microRNA 490-3P enhances the drug-resistance of human ovarian cancer cells. J Ovarian Res. 2014;7: 84.10.1186/s13048-014-0084-4

[69] He J, Yu JJ, Xu Q, Wang L, Zheng JZ, et al. Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. Autophagy. 2015: 0.10.1080/15548627.2015.1009781

[70] Bae HJ, Noh JH, Kim JK, Eun JW, Jung KH, et al. MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma. Oncogene. 2014;33(20): 2557-2567.10.1038/onc.2013.216

[71] Yan M, Chen C, Gong W, Yin Z, Zhou L, et al. miR-21-3p regulates cardiac hypertrophic response by targeting histone deacetylase-8. Cardiovasc Res. 2014.10.1093/cvr/cvu254

[72] Osanto S, Qin Y, Buermans HP, Berkers J, Lerut E, et al. Genome-wide microRNA expression analysis of clear cell renal cell carcinoma by next generation deep sequencing. PLoS One. 2012;7(6): e38298.10.1371/journal.pone.0038298

[73] Huang Y, Dai Y, Yang J, Chen T, Yin Y, et al. Microarray analysis of microRNA expression in renal clear cell carcinoma. Eur J Surg Oncol. 2009;35(10): 1119-1123.10.1016/j.ejso.2009.04.010

[74] Chow TF, Youssef YM, Lianidou E, Romaschin AD, Honey RJ, et al. Differential expression profiling of microRNAs and their potential involvement in renal cell carcinoma pathogenesis. Clin Biochem. 2010;43(1-2): 150-158.10.1016/j.clinbiochem.2009.07.020

[75] Youssef YM, White NM, Grigull J, Krizova A, Samy C, et al. Accurate molecular classification of kidney cancer subtypes using microRNA signature. Eur Urol. 2011;59(5): 721-730.10.1016/j.eururo.2011.01.004

[76] Yao Y, Ma J, Xue Y, Wang P, Li Z, et al. MiR-449a exerts tumor-suppressive functions in human glioblastoma by targeting Myc-associated zinc-finger protein. Mol Oncol. 2014.10.1016/j.molonc.2014.11.003

[77] Hales EC, Taub JW, & Matherly LH New insights into Notch1 regulation of the PI3K-AKT-mTOR1 signaling axis: targeted therapy of gamma-secretase inhibitor resistant T-cell acute lymphoblastic leukemia. Cell Signal. 2014;26(1): 149-161.10.1016/j.cellsig.2013.09.021

[78] Cheng Q, Yi B, Wang A, & Jiang X Exploring and exploiting the fundamental role of microRNAs in tumor pathogenesis. Onco Targets Ther. 2013;6: 1675-1684.10.2147/OTT.S52730

[79] Liu X, Gao Y, Lu Y, Zhang J, Li L, et al. Oncogenes associated with drug resistance in ovarian cancer. J Cancer Res Clin Oncol. 2015;141(3): 381-395.10.1007/s00432-014-1765-5

[80] Beaufort CM, Helmijr JC, Piskorz AM, Hoogstraat M, Ruigrok-Ritstier K, et al. Ovarian cancer cell line panel (OCCP): clinical importance of in vitro morphological subtypes. PLoS One. 2014;9(9): e103988.10.1371/journal.pone.0103988

[81] Behrens BC, Hamilton TC, Masuda H, Grotzinger KR, Whang-Peng J, et al. Characterization of a cis-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. Cancer Res. 1987;47(2): 414-418

[82] Hahne JC, Kurz A, Meyer SR, Dietl J, Engel JB, et al. Anti-tumour activity of phosphoinositide-3-kinase antagonist AEZS-126 in models of ovarian cancer. Arch Gynecol Obstet. 2015;291(1): 131-141.10.1007/s00404-014-3389-z

[83] Ali AY, Kim JY, Pelletier JF, Vanderhyden BC, Bachvarov DR, et al. Akt confers cisplatin chemoresistance in human gynecological carcinoma cells by modulating PPM1D stability. Mol Carcinog. 2014.10.1002/mc.22205

[84] Bao L, Jaramillo MC, Zhang Z, Zheng Y, Yao M, et al. Induction of autophagy contributes to cisplatin resistance in human ovarian cancer cells. Mol Med Rep. 2015;11(1): 91-98.10.3892/mmr.2014.2671

[85] Zhao JX, Liu H, Lv J, & Yang XJ Wortmannin enhances cisplatin-induced apoptosis in human ovarian cancer cells in vitro. Eur Rev Med Pharmacol Sci. 2014;18(17): 2428-2434

[86] Liu G, Du P, & Zhang Z Myeloid Differentiation Factor 88 Promotes Cisplatin Chemoresistance in Ovarian Cancer. Cell Biochem Biophys. 2014.10.1007/s12013-014-0294-9

[87] He G, Kuang J, Khokhar AR, & Siddik ZH The impact of S- and G2-checkpoint response on the fidelity of G1-arrest by cisplatin and its comparison to a non-cross-resistant platinum(IV) analog. Gynecol Oncol. 2011;122(2): 402-409.10.1016/j.ygyno.2011.04.034

[88] Hayakawa J, Ohmichi M, Kurachi H, Kanda Y, Hisamoto K, et al. Inhibition of BAD phosphorylation either at serine 112 via extracellular signal-regulated protein kinase cascade or at serine 136 via Akt cascade sensitizes human ovarian cancer cells to cisplatin. Cancer Res. 2000;60(21): 5988-5994

[89] Betito S & Cuvillier O Regulation by sphingosine 1-phosphate of Bax and Bad activities during apoptosis in a MEK-dependent manner. Biochem Biophys Res Commun. 2006;340(4): 1273-1277.10.1016/j.bbrc.2005.12.138

[90] Yang X, Fraser M, Moll UM, Basak A, & Tsang BK Akt-mediated cisplatin resistance in ovarian cancer: modulation of p53 action on caspase-dependent mitochondrial death pathway. Cancer Res. 2006;66(6): 3126-3136.10.1158/0008-5472.CAN-05-0425

[91] Fraser M, Bai T, & Tsang BK Akt promotes cisplatin resistance in human ovarian cancer cells through inhibition of p53 phosphorylation and nuclear function. Int J Cancer. 2008;122(3): 534-546.10.1002/ijc.23086

[92] Sasano T, Mabuchi S, Kuroda H, Kawano M, Matsumoto Y, et al. Preclinical Efficacy for AKT Targeting in Clear Cell Carcinoma of the Ovary. Mol Cancer Res. 2014.10.1158/1541-7786.MCR-14-0314

[93] Marsh Rde W, Rocha Lima CM, Levy DE, Mitchell EP, Rowland KM, Jr., et al. A phase II trial of perifosine in locally advanced, unresectable, or metastatic pancreatic adenocarcinoma. Am J Clin Oncol. 2007;30(1): 26-31.10.1097/01.coc.0000251235.46149.43

[94] Leighl NB, Dent S, Clemons M, Vandenberg TA, Tozer R, et al. A Phase 2 study of perifosine in advanced or metastatic breast cancer. Breast Cancer Res Treat. 2008;108(1): 87-92.10.1007/s10549-007-9584-x

[95] Snyder EL, Bailey D, Shipitsin M, Polyak K, & Loda M Identification of CD44v6(+)/CD24- breast carcinoma cells in primary human tumors by quantum dot-conjugated antibodies. Lab Invest. 2009;89(8): 857-866.10.1038/labinvest.2009.54

[96] Argiris A, Cohen E, Karrison T, Esparaz B, Mauer A, et al. A phase II trial of perifosine, an oral alkylphospholipid, in recurrent or metastatic head and neck cancer. Cancer Biol Ther. 2006;5(7): 766-770

[97] Knowling M, Blackstein M, Tozer R, Bramwell V, Dancey J, et al. A phase II study of perifosine (D-21226) in patients with previously untreated metastatic or locally advanced soft tissue sarcoma: A National Cancer Institute of Canada Clinical Trials Group trial. Invest New Drugs. 2006;24(5): 435-439.10.1007/s10637-006-6406-7

[98] Posadas EM, Gulley J, Arlen PM, Trout A, Parnes HL, et al. A phase II study of perifosine in androgen independent prostate cancer. Cancer Biol Ther. 2005;4(10): 1133-1137

[99] Ernst DS, Eisenhauer E, Wainman N, Davis M, Lohmann R, et al. Phase II study of perifosine in previously untreated patients with metastatic melanoma. Invest New Drugs. 2005;23(6): 569-575.10.1007/s10637-005-1157-4

[100] Vink SR, Schellens JH, Beijnen JH, Sindermann H, Engel J, et al. Phase I and pharmacokinetic study of combined treatment with perifosine and radiation in patients with advanced solid tumours. Radiother Oncol. 2006;80(2): 207-213.10.1016/j.radonc.2006.07.032

[101] Masoumi Moghaddam S, Amini A, Morris DL, & Pourgholami MH Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer. Cancer Metastasis Rev. 2012;31(1-2): 143-162.10.1007/s10555-011-9337-5

[102] Hahne JC, Okuducu AF, Sahin A, Fafeur V, Kiriakidis S, et al. The transcription factor ETS-1: its role in tumour development and strategies for its inhibition. Mini Rev Med Chem. 2008;8(11): 1095-1105

[103] Folkman J & Shing Y Angiogenesis. J Biol Chem. 1992;267(16): 10931-10934

[104] Risau W Mechanisms of angiogenesis. Nature. 1997;386(6626): 671-674.10.1038/386671a0

[105] Xia C, Meng Q, Cao Z, Shi X, & Jiang BH Regulation of angiogenesis and tumor growth by p110 alpha and AKT1 via VEGF expression. J Cell Physiol. 2006;209(1): 56-66.10.1002/jcp.20707

[106] Arbiser JL, Kau T, Konar M, Narra K, Ramchandran R, et al. Solenopsin, the alkaloidal component of the fire ant (Solenopsis invicta), is a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis. Blood. 2007;109(2): 560-565.10.1182/blood-2006-06-029934

[107] Jiang BH & Liu LZ PI3K/PTEN signaling in tumorigenesis and angiogenesis. Biochim Biophys Acta. 2008;1784(1): 150-158.10.1016/j.bbapap.2007.09.008

[108] Weis S, Cui J, Barnes L, & Cheresh D Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. J Cell Biol. 2004;167(2): 223-229.10.1083/jcb.200408130

[109] Zhang L, Yang N, Garcia JR, Mohamed A, Benencia F, et al. Generation of a syngeneic mouse model to study the effects of vascular endothelial growth factor in ovarian carcinoma. Am J Pathol. 2002;161(6): 2295-2309

[110] Li L, Wang L, Zhang W, Tang B, Zhang J, et al. Correlation of serum VEGF levels with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. Anticancer Res. 2004;24(3b): 1973-1979

[111] Ferrara N, Hillan KJ, & Novotny W Bevacizumab (Avastin), a humanized anti-VEGF monoclonal antibody for cancer therapy. Biochem Biophys Res Commun. 2005;333(2): 328-335.10.1016/j.bbrc.2005.05.132

[112] Hu L, Hofmann J, Zaloudek C, Ferrara N, Hamilton T, et al. Vascular endothelial growth factor immunoneutralization plus Paclitaxel markedly reduces tumor burden and ascites in athymic mouse model of ovarian cancer. Am J Pathol. 2002;161(5): 1917-1924.10.1016/S0002-9440(10)64467-7

[113] Mabuchi S, Terai Y, Morishige K, Tanabe-Kimura A, Sasaki H, et al. Maintenance treatment with bevacizumab prolongs survival in an in vivo ovarian cancer model. Clin Cancer Res. 2008;14(23): 7781-7789.10.1158/1078-0432.CCR-08-0243

[114] Errico A Breast cancer: combining bevacizumab with chemotherapy--from maintenance to second-line treatment. Nat Rev Clin Oncol. 2014;11(11): 621.10.1038/nrclinonc.2014.179

[115] Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. N Engl J Med. 2011;365(26): 2473-2483.10.1056/NEJMoa1104390

[116] Perren TJ, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, et al. A phase 3 trial of bevacizumab in ovarian cancer. N Engl J Med. 2011;365(26): 2484-2496.10.1056/NEJMoa1103799

[117] Aghajanian C, Blank SV, Goff BA, Judson PL, Teneriello MG, et al. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. J Clin Oncol. 2012;30(17): 2039-2045.10.1200/JCO.2012.42.0505

[118] Krupitskaya Y & Wakelee HA Ramucirumab, a fully human mAb to the transmembrane signaling tyrosine kinase VEGFR-2 for the potential treatment of cancer. Curr Opin Investig Drugs. 2009;10(6): 597-605

[119] Spratlin JL, Cohen RB, Eadens M, Gore L, Camidge DR, et al. Phase I pharmacologic and biologic study of ramucirumab (IMC-1121B), a fully human immunoglobulin G1 monoclonal antibody targeting the vascular endothelial growth factor receptor-2. J Clin Oncol. 2010;28(5): 780-787.10.1200/JCO.2009.23.7537

[120] Caligiuri MA Human natural killer cells. Blood. 2008;112(3): 461-469.10.1182/ blood-2007-09-077438

[121] Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, et al. Innate or adaptive immunity? The example of natural killer cells. Science. 2011;331(6013): 44-49.10.1126/ science.1198687

[122] Lanier LL Up on the tightrope: natural killer cell activation and inhibition. Nat Immunol. 2008;9(5): 495-502.10.1038/ni1581

[123] Moretta L, Biassoni R, Bottino C, Mingari MC, & Moretta A Human NK-cell receptors. Immunol Today. 2000;21(9): 420-422

[124] Lanier LL NK cell recognition. Annu Rev Immunol. 2005;23: 225-274.10.1146/annurev.immunol.23.021704.115526

[125] Dunn GP, Bruce AT, Ikeda H, Old LJ, & Schreiber RD Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3(11): 991-998.10.1038/ ni1102-991

[126] Orr MT & Lanier LL Natural killer cell education and tolerance. Cell. 2010;142(6): 847-856.10.1016/j.cell.2010.08.031

[127] Smyth MJ, Dunn GP, & Schreiber RD Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol. 2006;90: 1-50.10.1016/S0065-2776(06)90001-7

[128] Zhang Y, Wang X, Yang H, Liu H, Lu Y, et al. Kinase AKT controls innate immune cell development and function. Immunology. 2013;140(2): 143-152.10.1111/imm.12123

[129] Hawkins PT & Stephens LR PI3K signalling in inflammation. Biochim Biophys Acta. 2014.10.1016/j.bbalip.2014.12.006

[130] Soliman GA The role of mechanistic target of rapamycin (mTOR) complexes signaling in the immune responses. Nutrients. 2013;5(6): 2231-2257.10.3390/nu5062231

[131] Dituri F, Mazzocca A, Giannelli G, & Antonaci S PI3K functions in cancer progression, anticancer immunity and immune evasion by tumors. Clin Dev Immunol. 2011;2011: 947858.10.1155/2011/947858

[132] Bhoopathi P, Quinn BA, Gui Q, Shen XN, Grossman SR, et al. Pancreatic cancer-specific cell death induced in vivo by cytoplasmic-delivered polyinosine-polycytidylic acid. Cancer Res. 2014;74(21): 6224-6235.10.1158/0008-5472.CAN-14-0819

[133] Engel JB, Honig A, Kapp M, Hahne JC, Meyer SR, et al. Mechanisms of tumor immune escape in triple-negative breast cancers (TNBC) with and without mutated BRCA 1. Arch Gynecol Obstet. 2014;289(1): 141-147.10.1007/s00404-013-2922-9

[134] Noh KH, Kang TH, Kim JH, Pai SI, Lin KY, et al. Activation of Akt as a mechanism for tumor immune evasion. Mol Ther. 2009;17(3): 439-447.10.1038/mt.2008.255

[135] Bellucci R, Nguyen HN, Martin A, Heinrichs S, Schinzel AC, et al. Tyrosine kinase pathways modulate tumor susceptibility to natural killer cells. J Clin Invest. 2012;122(7): 2369-2383.10.1172/JCI58457

[136] Boutet P, Aguera-Gonzalez S, Atkinson S, Pennington CJ, Edwards DR, et al. Cutting edge: the metalloproteinase ADAM17/TNF-alpha-converting enzyme regulates proteolytic shedding of the MHC class I-related chain B protein. J Immunol. 2009;182(1): 49-53

[137] Waldhauer I, Goehlsdorf D, Gieseke F, Weinschenk T, Wittenbrink M, et al. Tumor-associated MICA is shed by ADAM proteases. Cancer Res. 2008;68(15): 6368-6376.10.1158/0008-5472.CAN-07-6768

[138] Okita R, Mougiakakos D, Ando T, Mao Y, Sarhan D, et al. HER2/HER3 signaling regulates NK cell-mediated cytotoxicity via MHC class I chain-related molecule A and B expression in human breast cancer cell lines. J Immunol. 2012;188(5): 2136-2145.10.4049/jimmunol.1102237