Drug-induced caspase 8 upregulation sensitises cisplatin-resistant ovarian carcinoma cells to rhTRAIL-induced apoptosis

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In ovarian cancer, the majority of tumours acquire drug resistance. Response rates to first-line platinum-based therapy are more than 80%, but most patients with advanced disease will finally relapse and die because of acquired drug resistance (Agarwal and Kaye, 2003). Chemoresistance is attributed to numerous mechanisms, which can be broadly divided into decreased DNA damage response via p53 and increased cell survival, mainly through defects in apoptosis (Werny and Morin, 2004). A meta-analysis showed that aberrant p53 status results in a worse 5-year survival for ovarian cancer patients (Crijns et al, 2003). Triggering apoptosis directly via the extrinsic apoptotic pathway might circumvent escape mechanisms developed by cancer cells. Especially the recombinant human (rh) form of the death ligand tumour necrosis factor-related apoptosis inducing ligand (TRAIL) is considered to be interesting for clinical use because of its ability to induce apoptosis in several types of cancer cell lines and xenografts (Wiley et al, 1995; Pitti et al, 1996; Ashkenazi et al, 2008). Preliminary data from a phase I trial with rhTRAIL showed no major toxicity (Herbst et al, 2010). TRAIL induces apoptosis by binding to death receptor 4 (DR4) and DR5 (Ashkenazi et al, 2008). Binding to these DRs causes receptor trimerisation and recruitment of the adaptor protein Fas-associated death domain (FADD). This in turn recruits caspase 8, resulting in the formation of the death-inducing signalling complex (DISC) (Peter, 2000). Binding of caspase 8 to the DISC causes its activation (Boatright et al, 2003), with subsequent activation of effector caspases 3, 6 and 7, which will execute apoptosis. The cellular FLICE-inhibitory protein (c-FLIP), which is vastly homologous to caspase 8 but lacks enzymatic activity, can also associate with the DISC, blocking activation of caspase 8 through competition for binding sites. It has also been stated that c-FLIP may function as an activator of caspase 8 under specific circumstances (Micheau et al, 2002; Boatright et al, 2004). In addition, the intrinsic apoptotic pathway can be activated by caspase 8 through cleavage of the BH3-only protein Bid that triggers perturbation of the mitochondria by Bax and Bak and finally activation of caspase 9 and effector caspases (Li et al, 1998). Numerous studies have demonstrated that drug resistance in cancer cells including ovarian cancer cells, could be prevented or overcome by combining rhTRAIL with chemotherapeutics (Mahalingam et al, 2009). In a previous study, we showed that cisplatin can sensitise ovarian cancer cells to rhTRAIL-induced apoptosis in vitro as well as in a bioluminescent ovarian cancer xenograft model (Duiker et al, 2009). The described mechanisms involved in modulation of ovarian cancer cells were established by comparison of cell lines with different background.
and sensitivity patterns, which impede establishment of causal relationships. Therefore, we investigated in an isogenic model of cisplatin resistance the molecular determinants for rhTRAIL sensitivity, the mechanism of synergy between cisplatin and rhTRAIL and the role of functional p53 in this synergy.

MATERIALS AND METHODS

Cell lines

The ovarian cancer cell lines A2780 and its five-fold cisplatin-resistant subline CP70, which carry wild-type and functional p53, were a kind gift from Dr Hamilton (Fox Chase Cancer Center, Philadelphia, PA, USA) (Godwin et al., 1992). Cisplatin resistance in CP70 can partly be explained by an increased DNA repair mechanism and a higher intracellular glutathione content (Johnson et al., 1994). Cells grew as monolayers in RPMI 1640 (Life Technologies Breda, the Netherlands), supplemented with 10% heat-inactivated fetal calf serum (FCS) (Bodinco BV, Alkmaar, the Netherlands) and 0.1% l-glutamine. Cell lines were cultured in a humidified atmosphere with 5% CO2.

Cytotoxicity assay

The microculture tetrazolium assay was used to measure cytotoxicity as described earlier (Duiker et al., 2009). Treatment consisted of continuous incubation with cisplatin (Pharmacochem BV, Haarlem, the Netherlands) or rhTRAIL (produced as described earlier (Ashkenazi et al., 1999)) for 96 h. The mean IC50 ± s.d. was determined in three experiments, each performed in quadruplicate.

Determination of apoptosis

In 96-well plates, cells were incubated with either rhTRAIL or cisplatin, or both. The cells were exposed to cisplatin for 4 h, washed with phosphate-buffered saline (PBS: 6.4 mM Na2HPO4; 1.5 mM KH2PO4; 0.14 mM NaCl; 2.7 mM KCl; pH = 7.2) twice and incubated in regular culture medium. At 20 h after administration of cisplatin, rhTRAIL was added for 4 h. Following rhTRAIL treatment, nuclear chromatin was stained with acridine orange to identify apoptosis by fluorescence microscopy.

SDS–polyacrylamide gel electrophoresis and western blotting

Cells were treated with rhTRAIL and/or cisplatin as described above. Treatment with the proteasome inhibitor MG132 (Calbiochem, Breda, the Netherlands) (0.5 μM) lasted for 24 h and rhTRAIL was added for the last 4 h. The caspase inhibitor I (zVAD) (Calbiochem) was added 2 h before MG132. Cycloheximide (CHX) (Sigma-Aldrich, Zwijndrecht, the Netherlands) (0.5 μM) was added 2 h before cisplatin, or both. The cells were exposed to cisplatin for 4 h, washed with phosphate-buffered saline (PBS: 6.4 mM Na2HPO4; 1.5 mM KH2PO4; 0.14 mM NaCl; 2.7 mM KCl; pH = 7.2) twice and incubated in regular culture medium. At 20 h after administration of cisplatin, rhTRAIL was added for 4 h. Following rhTRAIL treatment, nuclear chromatin was stained with acridine orange to identify apoptosis by fluorescence microscopy.

RNA interference and gene transfection

Small-interfering RNAs (siRNAs) were synthesised by Eurogentec (Seraing, Belgium). The double-stranded c-FLIP siRNA was 5′-GA GGUAGCGUGCGUGCGGdTdT-3′ (sense) and 5′-CCGACAGAC AGCUUACUC-dTdT-3′ (antisense). P53 siRNA 5′-GCAAUGAAG CGAGGGGCGCAUGdTdT-3′ (sense) and 5′-AUGGGCCUCGGUC AUGC-dTdT-3′ (anti-sense) was designed according to Martinez et al. (2002). Oligonucleotides specific for luciferase mRNA served as a negative control (Elbashir et al., 2001). Caspase 8 siRNA 5′-CUACCCAGAAGGGUAAUACCU-dTdT-3′ (sense) and 5′-AGGU AUACCUUCUGGUAGdTdT-3′ (anti-sense) was designed according to Chun et al. (2002) and negative control siRNA from Eurogentec was used. Subconfluent cells were incubated in unsupplemented Optimem medium and transfected with siRNA (up to 133 nM) using Oligofectamine reagent according to the manufacturer’s protocol (Invitrogen, Breda, the Netherlands). For the caspase 8 overexpression experiments, cells were transfected with pcDNA3-FLICE (a gift from J Borst) using FuGene. The next day, cells were treated with cisplatin and/or rhTRAIL as described above, collected and used for protein isolation, cytopsins, FACS experiments and/or an apoptosis assay. Cytopsins were immunohistochemically stained for caspase 8 as described earlier (Duiker et al., 2010).

Real-time RT–PCR

Total RNA was isolated by guanidine isothiocyanate–phenol–chloroform extraction with TRIzol (Invitrogen) and purified using the RNeasy mini Kit and on-column DNase I digestion (Qiagen, the Netherlands) according to the manufacturer’s instructions. Complementary DNA was synthesised from 1600 ng purified RNA as described by the manufacturer’s protocol (Life Technologies) using oligo (dT)11 primers and MMLV transcriptase. Real-time RT–PCR was performed in 96-wells plates on a MyiQ real-time detection system (Bio-Rad, Veenendaal, the Netherlands) with GAPDH as a housekeeping reference gene. Primer sequences of caspase 8 were 5′-GGAGCTGCTTCCGGGATTAGTT (forward) and 5′-GCAGTCTGATCGATGCTCCG (reverse) (Massey et al., 2001). The thermocycling programme used for each real-time RT–PCR consisted of an initial 3-min denaturation at 95 °C, followed by 40 cycles of 15-s denaturation at 95 °C, 20 s primer annealing at the primer specific Tm and 30-s fragment elongation at 72 °C. A melting curve was obtained at the end of each 40 cycles of amplification to determine the presence of a unique reaction product. To determine RT–PCR efficiency and initial starting quantity of the samples, a standard curve was generated using samples from a 1:3 dilution series of total starting cDNA.
Statistical analysis
All experiments were performed at least three times on different occasions. Analysis included double-sided non-paired Student’s t-test. A P-value <0.05 was considered significant.

RESULTS
Combination of cisplatin and rhTRAIL causes enhanced induction of apoptosis

A2780 was moderately sensitive to cisplatin treatment for 96 h, with an IC50 of 2.6 μM in a survival assay. The subline CP70 was five-fold resistant to cisplatin, with an IC50 of 14.7 μM (Figure 1A). The subline CP70 was rhTRAIL resistant, and A2780 was moderately sensitive, as IC50 was not reached using up to 0.25 μg ml⁻¹ in a survival assay for 96 h (Figure 1B). On the basis of previous data, cells were treated for 4 h with cisplatin, recovered for 20 h and treated with rhTRAIL for 4 h for the apoptosis assay (Duiker et al, 2009). Recombinant human TRAIL (0.25 μg ml⁻¹) induced moderate levels of apoptosis in A2780, ~20%, whereas CP70 was not sensitive to rhTRAIL. Combination of cisplatin with rhTRAIL enhanced apoptosis in both cell lines, with ~80% apoptosis in A2780 and ~40% apoptosis in CP70 (Figure 1C).

The resistant cell line CP70 has reduced caspase 8 protein levels

To determine which cellular characteristics could account for the different sensitivity patterns to rhTRAIL, key components of the TRAIL signalling pathway were analysed. Membrane expression of DR4 and DcR1 were almost undetectable in both cell lines. Decoy receptor 2 expression was similar, whereas DR5 was increased in CP70 (MFI = 120 ± 31) vs A2780 (MFI = 53 ± 26) (Figure 2A). Western blot analysis showed similar expression of FADD, c-FLIPs, c-FLIPL, caspases 9 and 3, Bid, Bax, Bak, Bcl-2 and Bcl-XL XIAP levels in A2780 and CP70. Remarkably, caspase 8 levels were lower in CP70 than in A2780 (Figure 2B).

Caspase 8 protein levels affect rhTRAIL-induced apoptosis

As caspase 8 protein levels are reduced in CP70 compared with A2780, we examined the importance of caspase 8 protein levels for rhTRAIL-sensitivity following cisplatin pre-treatment. Efficient downregulation of caspase 8 with siRNA strongly reduced apoptosis induction following treatment of A2780 and CP70 with rhTRAIL alone and combined with cisplatin (Figure 3A). In caspase 8 siRNA-transfected cells, combined treatment with cisplatin and rhTRAIL minimally induced caspases 8 and 3 activation, whereas in negative control siRNA-transfected cells, strong activation of both caspase 8 and 3 was observed (Figure 3B). It can be noticed that caspase 3 levels are also slightly upregulated following cisplatin in both cell lines (Figure 3B). A role for caspase 3 in rhTRAIL sensitisation can therefore not be excluded. However, the importance of caspase 8 levels was also demonstrated by transient upregulation of caspase 8 in CP70 that enhanced

Figure 1    Resistance to cisplatin causes cross-resistance to rhTRAIL. Combination therapy overcomes resistance. (A) Survival after 96 h exposure to 0–25 μM (A2780) or 0–100 μM (CP70) cisplatin and (B) survival after 96 h exposure to 0 μg ml⁻¹–0.25 μg ml⁻¹ rhTRAIL as measured by cytotoxicity assays. (C) To determine apoptosis induction, cells were treated for 4 h with cisplatin (2.5, 10 and 30 μM) after which cisplatin was washed away. Twenty hours later, the cells were treated for 4 h with 0.25 μg ml⁻¹ rhTRAIL. Apoptosis was determined with annexin V staining. Apoptosis in the combinations marked with * was significantly enhanced (P<0.05) over apoptosis after single-agent treatment. Data represent the mean ± s.d. of at least three independent experiments.
Apoptosis induction by rhTRAIL alone and by the rhTRAIL–cisplatin combination (Figures 3C and D).

Cisplatin increases caspase 8 mRNA levels

We investigated the mechanism causing reduced basal caspase 8 protein levels and cisplatin-induced caspase 8 upregulation in CP70. Basal caspase 8 mRNA levels were slightly higher in CP70 than in A2780. Exposure to cisplatin resulted in 1.5-fold induction of caspase 8 mRNA in both the cell lines (Figure 4A). Possible differences in mRNA stability over time and in response to cisplatin were determined using actinomycin D. Caspase 8 mRNA degradation in the absence or presence of cisplatin was not different between A2780 and CP70 ($P = 0.35$) (Figure 4B). These results show that cisplatin does not influence caspase 8 mRNA degradation, indicating that the induction of mRNA is caused by increased transcription.

Caspase 8 protein translation is reduced in CP70

The similar caspase 8 mRNA expression, whereas different basal caspase 8 protein levels were observed, suggest possible changes in caspase 8 protein translation or degradation in CP70. Cells were exposed to the proteasome inhibitor MG132 to test whether increased proteasomal degradation causes the reduced caspase 8 protein levels in CP70. Treatment with MG312 induced upregulation of active caspase 8, leading to concomitant synergy with rhTRAIL (Figure 4C). To prevent caspase 8 activation by MG132
treatment, cells were co-incubated with the caspase inhibitor zVAD for 24 h, which did not lead to a change in full-length caspase 8 levels (Figure 4D). Cycloheximide exposure for up to 24 h slightly affected caspase 8 levels in both cell lines, whereas caspase 8 degradation was not different between A2780 and CP70 (Figure 4E). These results show that not caspase 8 protein stability but rather a decreased translation of caspase 8 mRNA is causing the reduced caspase 8 expression in CP70.

Increased caspase 8 but not the caspase 8/c-FLIP ratio is involved in the response to cisplatin and rhTRAIL in CP70

Difference in caspase 8/c-FLIP ratio between A2780 and CP70 might contribute to resistance in CP70. However, efficient down-regulation of both c-FLIPp and c-FLIP, with siRNA did not lead to increased apoptosis after either treatment regimen, and did not affect caspase 8 cleavage (Figure 5A). Downregulation even led to a significant decrease in apoptosis in CP70 (Figure 5B). These results indicate that solely the elevated caspase 8 level is involved in the onset of apoptosis after combination treatment in CP70. Moreover, c-FLIP even promotes caspase 8 activation in these cells.

P53 causes cisplatin-induced DR5 expression, but is not involved in rhTRAIL sensitisation

As p53-induced upregulation of DR5 is frequently described to be instrumental in the synergistic effect between chemotherapeutics and rhTRAIL (Wu et al., 2004), we asked whether the synergistic effect of cisplatin and rhTRAIL was p53 dependent. The tumor suppressor protein p53 was efficiently and functionally down-regulated using siRNA in A2780 and CP70 as shown by the decreased expression of p21 (Waf1/Cip1), a transcriptional target of p53 (Figure 6A). In response to cisplatin, p53 levels rose slightly in p53-suppressed cells, but the levels remained far below those in the untreated luciferase siRNA-transfected cells. Apoptosis induction (Figure 6B) and activation of caspases 8, 9 and 3 (Figure 6C) were not affected by p53 siRNA. Following p53 downregulation, basal DR5 membrane expression maintained unchanged, whereas...

Figure 3 Caspase 8 downregulation inhibits cisplatin and rhTRAIL-induced apoptosis, whereas caspase 8 upregulation augmented apoptosis induction. A2780 and CP70 were transfected with siRNA against caspase 8 or negative control siRNA (A and B). The CP70 cells were transiently transfected with a caspase 8 construct (C and D). At 48 h after transfection, cells were treated with 30 μM cisplatin or medium control for 4 h, after which all cells were washed with PBS. Following 16 h of recovery, cells were treated with 0.25 μg ml⁻¹ rhTRAIL or medium control for 4 h. (A) Apoptosis induction was determined by acridine orange staining. Caspase 8 siRNA strongly reduced apoptosis induction by rhTRAIL and cisplatin in both cell lines. (B) Caspase 8 and 3 expression was determined using western blot analysis. The exposure time of the caspase 8 blot of CP70 was increased compared with the blot of A2780. β-Actin serves as a loading control. The blots are representative for at least three independent experiments. (C) Cytospins of CP70 cells (1,2) were generated and stained for caspase 8 and show increased caspase 8 levels following transfection (2a+b) compared with untransfected cells (1a+b). (D) Apoptosis induction following cisplatin and rhTRAIL was determined by acridine orange staining. Caspase 8 upregulation increased apoptosis induction by rhTRAIL alone and by rhTRAIL in combination with cisplatin.
Caspase 8 mRNA levels do not differ significantly between A2780 and CP70. The central role of caspase 8 was further indicated by our observation that cisplatin-induced DR5 upregulation was not an important sensitising factor. As caspase 8 mRNA levels do not differ significantly between A2780 and CP70, the low caspase 8 protein levels in CP70 are likely to be caused by decreased translation of caspase 8 protein rather than decreased transcription through methylation or genetic alterations that occur in neuroblastoma (Teitz et al., 2000; Takita et al., 2001) and in other solid tumours (Kim et al., 2003; Soung et al., 2005). Increased caspase 8 protein degradation in CP70 was ruled out, as the effect of inhibition of protein synthesis and proteasomal degradation on caspase 8 levels was not different between A2780 and CP70.

Translational and post-translational modifications are important factors of the expression levels and activity of key proteins in the regulation of cell survival and apoptosis such as p53

cisplatin-induced DR5 membrane expression was effectively suppressed in A2780 and CP70 (Figure 6D). These results show that p53-dependent upregulation of DR5 is not involved in the synergy between cisplatin and rhTRAIL. In addition, DISC-IP using a DR5 antibody following cisplatin treatment showed that DISC formation is not impeded in CP70 (data not shown).

**DISCUSSION**

In the present study, we show that the cellular caspase 8 protein level is an important determinant of sensitivity to rhTRAIL-induced apoptosis in an isogenic ovarian cancer cell line model of acquired cisplatin resistance. Combination of cisplatin and rhTRAIL effectively induced apoptosis, with cisplatin-induced caspase 8 protein expression being the key factor of sensitisation to rhTRAIL in CP70.
siRNA c-FLIP

2001; Spierings et al in the response to different chemotherapeutic drugs (Fulda dependent and -independent activation of caspase 8 was involved death, conflicting reports have been published. Death receptor- and the extrinsic apoptotic pathway in chemotherapy-induced cell death (Abedini et al (Zhang et al (Fraser et al (Xu, 2003), p73 (Ozaki et al, 2005), XIAP and APAF-1 (Holcik and Sonenberg, 2005). Recently, attention has been drawn to micro-RNAs (miRNAs) as important regulators of translation and mRNA stability (Kent and Mendell, 2006). Dereglulation of several miRNAs was described for ovarian cancer, and an important role for miRNAs in cisplatin and TRAIL resistance was shown in ovarian and lung cancer, respectively (Garofalo et al, 2008; Yang et al, 2008). In melanoma cells, an inducible post-translational modification of mRNA contributed to TRAIL resistance, in which cytosolic proteins could suppress DR5 protein expression by binding to the 3'-untranslated region of DR5-mRNA (Zhang et al, 2004). These studies support our hypothesis that a post-transcriptional mechanism is involved in the low expression of caspase 8 in CP70. The fact that most prominently in CP70, caspase 8 translation could be enhanced by cisplatin exposure argues in favour of a reversible block of translation. Low caspase 8 levels were previously shown to contribute to rhTRAIL resistance in several cell line models and tumours (Hopkins-Donaldson et al, 2000; Zhang et al, 2005). Regarding the role of caspase 8 and the extrinsic apoptotic pathway in chemotherapy-induced cell death, conflicting reports have been published. Death receptor-dependent and -independent activation of caspase 8 was involved in the response to different chemotherapeutic drugs (Fulda et al, 2001; Spierings et al, 2003; Longley et al, 2006). In addition, resistance to these drugs through defects in the extrinsic pathway further supported the involvement of this pathway in chemotherapy-induced cell death (Abedini et al, 2004; Longley et al, 2006).

Besides the differential expression of caspase 8, we found no other proteins that could account for the difference in rhTRAIL sensitivity between A2780 and CP70. As caspase 3 was slightly upregulated in both cell lines following cisplatin, a role for caspase 3 and hence also for XIAP can not be completely ruled out (Fraser et al, 2003). However, basal caspase 3 levels did not differ between the two cell lines, excluding caspase 3 as a determinant for the difference in rhTRAIL sensitivity. A reduced caspase 8/c-FLIP ratio can result in resistance to DR signalling (Mitsiades et al, 2002; Baumler et al, 2003), whereas an increased caspase 8/c-FLIP ratio in the DISC can cause sensitisation to rhTRAIL by chemotherapeutics (Lacour et al, 2003; Ganten et al, 2004). In our cell lines, downregulation of c-FLIP did not increase apoptosis induction. In the resistant CP70, downregulation of c-FLIP even resulted in a significant drop in apoptosis levels after exposure to cisplatin and rhTRAIL. This suggests that c-FLIP functions as a pro-apoptotic protein in CP70 and that the caspase 8 level is the most important determinant for rhTRAIL sensitivity. Although extensive literature exists on the anti-apoptotic function of c-FLIP, a role of c-FLIP in activation of caspase 8 was also reported (Micheau et al, 2002; Boaright et al, 2004). It is postulated that the c-FLIP(32 kDa) concentration at the DISC determines whether caspase 8 activation or inhibition occurs (Boaright et al, 2004; Peter, 2004). Thus, it can be reasoned that the caspase 8-activating function of FLIP applies to A2780 and CP70, especially when low caspase 8 levels are present and heterodimerisation can occur more easily than homodimerisation.

Upregulation of DR4 or DR5, which can occur in a p53-dependent or -independent manner in response to chemotherapeutics (Wu et al, 1997; Sheikh et al, 1998), is often described as a key event in the synergistic effect between chemotherapeutics and rhTRAIL (Wu et al, 2004). In this study, we show that the increase of DRs after chemotherapy treatment might just be an epiphenomenon, instead of a key event in modulation of rhTRAIL-induced cell death by chemotherapeutics. Downregulation of p53 did not affect apoptosis induction in both cell lines, whereas the substantial increase of DR5 after treatment with cisplatin was almost completely abrogated by p53 downregulation. It has been shown that p53 is required for cisplatin-induced apoptosis either via p53-dependent ubiquitination of FLIP or through p53-induced XIAP and Akt downregulation when used as a single agent (Fraser et al, 2003). However, downregulation of XIAP and Akt and inhibition of caspase 3 were not observed in our cell lines, indicating that the mechanism by which cisplatin induces apoptosis is different from that described for other cell lines. In conclusion, the present study demonstrates that differences in the expression of extrinsic and intrinsic apoptosis pathways determine the sensitivity of two ovarian cancer cell lines to chemotherapy and rhTRAIL. These results underline the importance of identifying drug combination strategies to increase the therapeutic efficacy of chemotherapy in ovarian cancer.

Figure 5 The caspase 8/c-FLIP ratio is not involved in resistance to rhTRAIL. Cells were transfected with siRNA against c-FLIP or luciferase. The next day, cells were exposed to cisplatin for 4 h, after which all cells including those conditions left unexposed were washed. The following day after 4 h incubation with or without 0.25 μg/ml rhTRAIL cell lysates were made (A). Cellular FLICE-inhibitory protein (c-FLIP) and caspase 8 cleavage was determined with western blot analysis. The exposure time of the caspase 8 blot of CP70 was increased compared with the blot of A2780. The blots are representative for at least three independent experiments. (B) A small fraction of the same cell suspension used for western blot was plated in a 96-wells plate and apoptosis induction was assessed by annexin V staining. Differences in apoptosis were statistically significant (P< 0.05).
These results may explain why CP70 has reduced caspase 8 levels compared with A2780. However, in our experiments we have chosen to use cisplatin in a short-treatment setting. This was followed by a short treatment with rhTRAIL, and we have demonstrated that upon combination of cisplatin and rhTRAIL, p53 is no longer required for apoptosis induction.

Conclusively, these results show that cisplatin enhances rhTRAIL sensitivity in both cisplatin-sensitive and -resistant cells. Induction of caspase 8 protein expression is the key factor of rhTRAIL sensitisation.

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REFERENCES

Abedini MR, Muller EJ, Brun J, Bergeron R, Gray DA, Tsang BK (2008) Cisplatin induces p53-dependent FLICE-like inhibitory protein ubiquitination in ovarian cancer cells. Cancer Res 68: 4511–4517

Abedini MR, Qiu Q, Yan X, Tsang BK (2004) Possible role of FLICE-like inhibitory protein (FLIP) in chemoresistant ovarian cancer cells in vitro. Oncogene 23: 6997–7004

Agarwal R, Kaye SB (2003) Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nat Rev Cancer 3: 502–516

Ashkenazi A, Holland P, Eckhardt SG (2008) Ligand-based targeting of apoptosis in the potential of recombinant human apoptosis ligand 2 tumor necrosis factor-related apoptosis-inducing ligand (rApo2L/TRAIL). J Clin Oncol 26: 3621–3630

Ashkenazi A, Pai RC, Fong S, Leung S, Lawrence DA, Marsters SA, Blackie C, Chang L, McMurtrey AE, Hebert A, DeForge L, Koumenis IL, Lewis D, Harris L, Bussiere J, Koeppen H, Shahrorkh Z, Schwall RH (1999) Safety and antitumor activity of recombinant soluble Apo2 ligand. J Clin Invest 104: 155–162

Baumler C, Duan F, Onel K, Rapaport B, Jhanwar S, Offit K, Elkon KB (2003) Inactivation of caspase-8 gene in colorectal carcinomas. Gastroenterology 125: 708–715

Baumler C, Duan F, Onel K, Rapaport B, Jhanwar S, Offit K, Elkon KB (2003) Possible role of FLICE-like inhibitory protein (FLIP) in chemoresistant ovarian cancer cells in vitro. Oncogene 23: 6997–7004

Bjana S, Vickers SJ, Almeda A, Veal A (2002) Androgens enhance TRAIL-induced apoptosis by chemotherapeutic drugs.

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Wu GS, Burns TF, McDonald III ER, Jiang W, Meng R, Krantz ID, Kao G, Gan DD, Zhou JY, Muschel R, Hamilton SR, Spinner NB, Markowitz S, Wu G, El-Deiry WS (1997) KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. Nat Genet 17: 141 – 143

Wu XX, Ogawa O, Kakehi Y (2004) TRAIL and chemotherapeutic drugs in cancer therapy. Vitam Horm 67: 365 – 383

Xu Y (2003) Regulation of p53 responses by post-translational modifications. Cell Death Differ 10: 400 – 403

Yang H, Kong W, He L, Zhao JJ, O’Donnell JD, Wang J, Wenham RM, Coppola D, Kruk PA, Nicosia SV, Cheng JQ (2008) MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 68: 425 – 433

Zhang L, Zhu H, Teraishi F, Davis JJ, Guo W, Fan Z, Fang B (2005) Accelerated degradation of caspase-8 protein correlates with TRAIL resistance in a DLD1 human colon cancer cell line. Neoplasia 7: 594 – 602

Zhang XY, Zhang XD, Borrow JM, Nguyen T, Hersey P (2004) Translational control of tumor necrosis factor-related apoptosis-inducing ligand death receptor expression in melanoma cells. J Biol Chem 279: 10606 – 10614