Mechanisms of Action of DNA-Damaging Anticancer Drugs in Treatment of Carcinomas: Is Acute Apoptosis an "Off-Target" Effect?

Aleksandra Mandic Havelka, Maria Berndtsson, Maria Hägg Olofsson, Maria C. Shoshan and Stig Linder*

Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska, Institute and Hospital, S-171 76 Stockholm, Sweden

Abstract: DNA damage induces apoptosis of cells of hematological origin. Apoptosis is also widely believed to be the major antiproliferative mechanism of DNA damaging anticancer drugs in other cell types, and a large number of laboratories have studied drug-induced acute apoptosis (within 24 hours) of carcinoma cells. It is, however, often overlooked that induction of apoptosis of carcinoma cells generally requires drug concentrations that are at least one order of magnitude higher than those required for loss of clonogenicity. This is true for different DNA damaging drugs such as cisplatin, doxorubicin and camptothecin. We here discuss apoptosis induction by DNA damaging agents using cisplatin as an example. Recent studies have shown that cisplatin induces caspase activation in enucleated cells (cytoplasts lacking a cell nucleus). Cisplatin-induced apoptosis in both cells and cytoplasts is associated with rapid induction of cellular reactive oxygen species and increases in $[Ca^{2+}]_i$. Cisplatin has also been reported to induce clustering of Fas/CD95 in the plasma membrane. Available data suggest that the primary responses to cisplatin-induced DNA damage are induction of long-term growth arrest ("premature cell senescence") and mitotic catastrophe, whereas acute apoptosis may be due to "off-target effects" not necessarily involving DNA damage.

INTRODUCTION

Despite major efforts by the pharmaceutical industry to develop target-specific anticancer drugs, DNA damaging drugs remain cornerstone agents for cancer therapy. Topoisomerase I and II inhibitors, alkylating agents and platinum compounds all induce robust clinical responses in many cancers, without, however, generally being curative. DNA damage-inducing drugs induce complex phenotypic effects on cells, effects that are both cell context-dependent, drug-dependent and drug concentration-dependent. The perhaps most well studied anti-proliferative effect of this class of agents is apoptosis. In addition, a number of other effects are provoked, including induction of premature senescence, apoptosis and necrosis [1]. The purpose of this article is to discuss the mechanisms of action of DNA damaging drugs at the cellular level, based on cisplatin as an example.

Cisplatin is one of the most potent anticancer drugs and one of the very few which can be curative. Cisplatin is used in the treatment of several cancers, such as ovarian, testicular, cervix, head-and-neck, small-cell lung cancer and urinary bladder cancers [2]. Cisplatin undergoes aquation in the intracellular environment characterized by a low chloride ion concentration. The aquated compound forms covalent bonds to the N7 positions of DNA purines resulting in intra- or interstrand crosslinks [3-5]. The main DNA repair pathway for these adducts is the nucleotide-excision-repair pathway (NER) [6].

*Address correspondence to this author at Cancer Canter Karolinska, R8:00, Department of Oncology and Pathology, Karolinska, Institute and Hospital, S-171 76 Stockholm, Sweden; Tel: +46-8-51772452; Fax: +46-8-339031; E-mail: Stig.Linder@cck.ki.se

CISPLATIN INDUCES APOPTOTIC SIGNALING

It was initially believed that cisplatin cytotoxicity was due to inhibition of DNA synthesis [7]. The drug was later shown to induce internucleosomal cleavage of chromatin, consistent with apoptosis [8]. This observation has generated considerable interest with regard to the mechanism of action of cisplatin and with regard to the mechanisms of resistance to the compound. Cisplatin has also been frequently used as a model agent for induction of DNA damage-induced apoptosis *in vitro*.

At the concentrations utilized in the literature, cisplatin induces apoptosis within 24 – 48 hours, a time frame which is convenient in signal transduction experiments. DNA damage signaling mechanisms have been postulated to induce apoptotic signaling. Most studies point to an interplay between regulators and effectors of the DNA-repair and apoptosis-execution machineries. Following DNA damage, the p53 tumor-suppressor protein is activated and subsequently trans-activates different sets of downstream target genes. Cisplatin can also induce the accumulation of the p53-related gene p73 in a c-ABL dependent manner [9]. Interestingly, p73 induction does not occur in mismatch repair deficient cells [9]. Resistance to cisplatin correlates with the presence of mutant p53 in the National Cancer Institute (NCI) panel of 60 human tumor cell lines [10]. Reconstitution of mutant tumor cell lines with wild-type p53 increases sensitivity [11,12]. In contrast to these findings which strongly suggest that p53 is a resistance factor for cisplatin, other studies show no or even negative correlation between p53 status and sensitivity to cisplatin [13], see [3].

p53 has been shown to control the expression of the death domain-containing adaptor protein PIDD that, together with the adaptor protein RAIDD, activates procaspase-2

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[14]. Caspase-2 acts upstream of mitochondrial release of cytochrome *c* [15]. Caspase-2 is capable of cleaving the proapototic Bcl-2 family member Bid [16]. Caspase-2 activation appears to be a response to severe stress, occurring at high cisplatin exposures and leading to rapid cell death [17]. Other mechanisms of DNA damage-induced apoptosis have been described, such as induction of E2F1 [18]. It has also been reported that DNA damage induces E2F1 acetylation, which is required for its recruitment onto apoptotic gene promoters [19].

The MAPK family members c-JUN N-terminal kinase (JNK) and p38 are strongly activated in response to various stress signals, including exposure to cisplatin and other anticancer drugs, exposure to ionizing and ultraviolet irradiation to and hyperosmotic stress [20]. Cisplatin induces sustained activation of p38 MAPK in sensitive ovarian carcinoma cells, whereas activation is only transient (< 3 hours) in resistant cells [21]. Several studies have suggested that the JNK pathway contributes to cisplatin-induced apoptosis [21-24]. In other studies cisplatin-induced JNK activation signals prosurvival [25,26]. These disparate observations might reflect the nature of different cell types and specificity or transient versus persistent activation patterns, respectively [27].

The Bcl-2 family proteins are central regulators of apoptosis [28] and a number of studies have implicated these proteins in cisplatin sensitivity [29]. There are, however, interesting divergences. Beale and coworkers [30] showed that expression of Bcl-2 led to cisplatin resistance when assayed at 48 hours of exposure. However, Bcl-2 conferred an increase in sensitivity when assayed at 96 hours, and did not affect sensitivity when assayed as clonogenic survival. Other investigators have made similar observations [31-34] and these results have led to questions with regard to the relevance of apoptosis as a major factor in conferring sensitivity of carcinoma cells to anticancer drugs. As discussed by Brown and Wouters [35], apoptosis is measured in short term assays where the rate of cell death is very important. When clonogenic survival is used to assess cell death, apoptosis-regulating genes such as p53 and Bcl-2 play little or no role in the sensitivity to anticancer drugs and radiation.

APOPTOSIS VERSUS SENESCENCE AND MITOTIC CATASTROPHE

Apoptosis is not the only anti-proliferative response elicited by cisplatin. The drug has also been shown to induce premature senescence [36] and mitotic catastrophe [37]. Mitotic catastrophe is a form of cell death resulting from abnormal mitosis and leading to the formation of interphase cells with multiple micronuclei. Premature senescence is currently thought to be related to replicative senescence [38]. Proliferation arrest associated with replicative senescence is mediated by signaling pathways involving ATM (Ataxia telangiectasia mutated) and p53. The p53 pathway is thought to be activated by telomere uncapping [39,40]. The same signaling mechanisms may be involved in premature senescence induced by DNA damaging drugs [41].

The saying that "if you don´t get older you are dead" is true both for humans and for cells: apoptosis and senescence are obviously mutually exclusive cellular outcomes. The factors that decide whether cisplatin and other DNA damaging agents will trigger apoptosis or senescence are not clearly understood. In one scenario, senescence and apoptosis represent graded responses to increasing DNA damage. In more complex scenarios, cisplatin induces senescence and apoptosis by different (or partially different) mechanisms.

The concentrations used to induce apoptosis are much higher than those required to inhibit clonogenic outgrowth (10 - 50 μM for induction of apoptosis versus approximately 0.5 - 1 μM for loss of clonogenicity, see [42]). We have suggested that inhibition of clonogenic outgrowth by cisplatin is primarily due to induction of senescence [42]. Our data show that premature senescence, but not apoptosis, is induced by the same (low) concentration range of cisplatin that is sufficient to induce DNA damage [42]. We also find that cisplatin-induced apoptosis and senescence differ in sensitivity to pharmacological inhibitors: whereas apoptosis is inhibited both by the calcium chelator BAPTA-AM and by the superoxide scavenger Tiron, senescence is insensitive to both these agents [42].

ARE REPAIR-DEFECTIVE CELLS MORE SENSITIVE TO CISPLATIN-INDUCED APOPTOSIS?

A large number of studies have demonstrated that DNA repair deficient cells show increased sensitivity to cisplatin. These studies were generally performed using clonogenic assays and out-growth assays (typically 3 day assays) and drug concentrations around 1 μM (see [43,44] and references therein). Deficiencies in the NER pathway, including the xeroderma pigmentosum (*XP*) and excision repair cross-complementing (*ERCC*) gene products, were found to be associated with cisplatin sensitivity [45-47]. ERCC1 was expressed at 2.6-fold higher levels in tumors resistant to cisplatin [48], and down-regulation of ERCC1 lead to sensitization to cisplatin [47].

Treatment with cisplatin has been reported to increase phosphorylation of ATM at Ser-1981 [42,49], but also to decrease it [50]. ATM phosphorylation was not observed in cells defective in the NER pathway [49]. This defect is expected to lead to impaired signaling to downstream mediators such as p53 and CHK1/CHK2 and, paradoxically, to impaired apoptosis. The literature is not consistent with regard to whether DNA repair-deficient cells are more or less sensitive to cisplatin-induced apoptosis (see [49,51]). It is, however, often stated that defects in DNA repair will lead to impaired apoptosis, and that repair defects therefore will drive oncogenesis [52]. This view is inconsistent with the idea that DNA damaging drugs induce apoptosis.

CONTRIBUTION OF A NUCLEUS-INDEPENDENT MECHANISM TO APOPTOSIS

The use of high cisplatin concentrations to induce apoptosis is not unproblematic. In analogy with the use of pharmacological inhibitors at high concentrations, it must be considered whether such high concentrations lead to "off-target" effects, i.e. effects that are independent of damage to the pri-mary target, nuclear DNA. We originally reported that cisplatin induces apoptotic responses in enucleated cytoplasts [53]. Similar findings have been reported by other groups using cisplatin [54] and oxaliplatin [55]. It seems unlikely that the cytoplasmic signaling mechanism is an arti-

fact that can be triggered by any stimulus: other studies have identified a number of other pro-apoptotic agents which do not induce caspase-activation in cytoplasts [56]. On the contrary, the cytoplasmic signaling mechanism is likely to be important for apoptosis induction by cisplatin. First, the dose-response of activation of caspase-3 in cytoplasts and intact cells is similar [42,54]. Secondly, apoptotic signaling in cytoplasts and intact cells is inhibited by the same pharmacological inhibitors (see below).

ON THE NATURE OF CYTOPLASMIC TARGETS OF CISPLATIN

What could be the nature of a cytoplasmic target of cisplatin? First of all, one has to consider whether DNA necessarily is the target macromolecule. It is well known that cisplatin reacts with proteins due to its electrophilicity toward methionine, cysteine and histidine [57,58]. In fact, protein adducts have been calculated to be more abundant than DNA adducts: 25 μM cisplatin induces 5 x 10^4 DNA adducts and > 10^6 protein adducts per cell [59]. Cisplatin has also been shown to complex specifically with phosphatidylserine in plasma membranes [60]. Although this massive production of protein adducts could possibly trigger signaling events and could contribute to the anti-proliferative activities of cisplatin, mechanisms not involving DNA have rarely been considered.

An important clue to the nature of the mechanism of apoptosis induction by cisplatin is the time kinetics of signaling induced by this agent. Cisplatin induces rapid increases in cellular superoxide levels [42] and increases in $Ca^{2+}_{(i)}$ [61]. We showed that increases in $Ca^{2+}_{(i)}$ are followed by activation of calpain and cleavage of Bid [53,61]. These signaling events are not expected to result from DNA damage-induced signaling but are likely to be parallel phenomena. We have shown that the Ca^{2+} chelator BAPTA-AM inhibits caspase-3 activation both in intact cells and in enucleated cytoplasts [53] and that ROS scavengers inhibit apoptosis in cells and cytoplasts [42]. We therefore hypothesize that cisplatin induces ROS production in cells, that this will lead to oxidation of the redox-sensitive channels in the ER, and release of Ca^{2+} to the cytoplasm (Fig. **1**). We and others have found that cisplatin induces expression of the ER chaperones GRP78 and GRP94 and caspase-12 cleavage [53,62]. Cisplatin-induced apoptosis may therefore at least in part be due to an ER stress response.

An alternative, or additional, non-nuclear mechanism of apoptosis induction involves caspase-8. Cisplatin induces clustering of Fas receptors and interaction of the receptor with FADD [63] (Fig. **1**). Overexpression of FADD sensitizes tumor cells to cisplatin-induced cell death and down-regulation of FADD decreases drug sensitivity. Cisplatin activates acid sphingomyelinase resulting in increases in

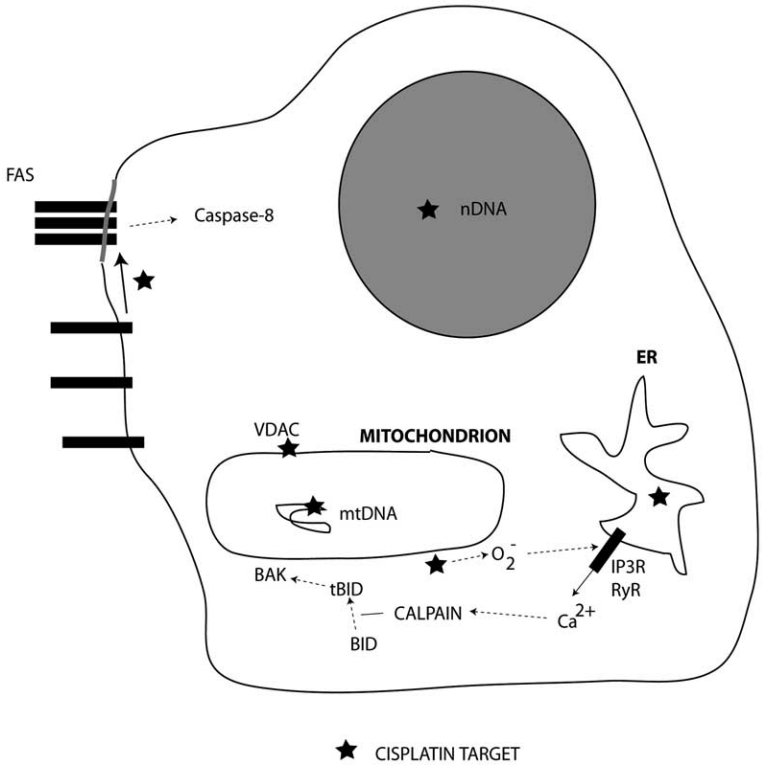

Fig. (1). Potential cytoplasmic mechanisms leading to cisplatin-induced apoptosis.

Cisplatin induces cellular superoxide (O^{2-}). The source of superoxide is not known (in the figure, superoxide is assumed to originate from mitochondrial respiration; there are other possibilities such as NADPH oxidases [69]). Superoxide is hypothesized to induce release of $Ca^{2+}_{(i)}$ from the ER [42] and to activation of calpain and cleavage of Bid [53,61]. Cisplatin activates acid sphingomyelinase, resulting in increases in ceramide and resulting in redistribution of CD95 into plasma membrane rafts [64]. Clustering of Fas receptor leads to interaction of the receptor with FADD and activation of caspase-8 [63]. Mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA to various DNA damaging agents [65] and cells that lack mitochondrial DNA were reported resistant to cisplatin [54].

ceramide, resulting in redistribution of CD95 into plasma membrane rafts [64].

Another candidate cytoplasmic target is the mitochondrion (Fig. **1**). Mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA to various DNA damaging agents [65] and cisplatin induces at least two orders of magnitude higher adduct levels in mtDNA compared to nuclear DNA [54]. It was also recently reported that rho^0 cells (that lack mitochondrial DNA) are resistant to cisplatin [54]. How damage to mtDNA is sensed and a signal transduced to the apoptosis machinery is not known; no such systems have been described. It should also be pointed out that rho^0 cells may overexpress MnSOD and that such cells can be resistant to various agents known to induce ROS (menadione, paraquat and doxorubicin) [66]. The resistance of rho^0 cells to cisplatin could therefore be a consequence of resistance to oxidative stress.

It has also been reported that addition of cisplatin to isolated mitochondria induces release of cytochrome c within minutes and to mitochondrial disruption within a few hours [54]. Whether these events are due to increases in cisplatin-DNA adducts is not known, and it is difficult to see how damage to mtDNA leads to cytochrome c release in a cell free system. Our studies show that cisplatin induces alterations of mitochondrial membrane potential after several hours in melanoma cell lines (our unpublished data).

CISPLATIN ACTIVITY *IN VIVO*

Cisplatin is generally given at doses between 50 – 100 mg/m^2, corresponding to approximately 1 – 2 mg/kg. Whether such concentration of cisplatin induce apoptosis *in vivo* is not clear. In one report, injection of cisplatin into mice using a dose of 1 mg/kg did not result in detectable apoptosis, but instead led to an increase in binucleated cells with micronuclei [67].

We have observed induction of apoptosis *in vivo*, as evidenced by caspase-cleaved cytokeratin-18 fragments in serum, in mice treated with 5 mg/kg cisplatin (MHO and AMH, unpublished data). Whether this is a clinically relevant concentration is unclear.

OTHER DNA DAMAGING ANTICANCER DRUGS

An obvious question is whether the arguments made here regarding cisplatin also are true for other DNA damaging agents. We believe that they often do. Doxorubicin has been shown to induce different cellular responses at different concentrations [68]. Examination of the literature shows that doxorubicin is usually given at dose of 1 μg/ml for induction of apoptosis (corresponding to approximately 1.7 μM). Inspection of the NCI data base (60 cell line screeen; www.dtp.nci.nih.gov) shows that the IC50 of doxorubicin in a 6 day outgrowth assay is approximately 10 nM. The IC50 of the topoisomerase-1 inhibitor camptothecin in a 6 day outgrowth assay is also approximately 10 nM, but the agent is used at concentration of 1 μM or higher for induction of apoptosis in the literature. It therefore seems that investigators, knowing that these various agents kill cells by apoptosis, use the required amount of drug to achieve the desired outcome.

CONCLUSIONS

We argue that it should be seriously considered that the primary response to DNA damage-inducing agents may not be apoptosis but induction of premature cell senescence and or mitotic catastrophe. In contrast, induction of acute apoptosis of carcinoma cells by these agents, studied in hundreds of publications, may due to "off-target effects" induced by high drug concentrations.

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REFERENCES

[1] Roninson, I. B.; Broude, E. V.; Chang, B. D. *Drug Resist. Updat*., **2001**, *4*, 303.
[2] Boulikas, T.; Vougiouka, M. *Oncol. Rep*., **2004**, *11*, 559.
[3] Wang, D.; Lippard, S. J. *Nat. Rev. Drug Discov*., **2005**, *4*, 307.
[4] Brabec, V.; Kasparkova, J. *Drug Resist. Updat*., **2002**, *5*, 147.
[5] Jamieson, E. R.; Lippard, S. *Chem. Rev*., **1999**, *99*, 2467.
[6] Zamble, D. B.; Lippard, S. J. *Trends Biochem. Sci*., **1995**, *20*, 435.
[7] Harder, H. C.; Rosenberg, B. *Int. J. Cancer*, **1970**, *6*, 207.
[8] Sorenson, C. M.; Barry, M. A.; Eastman, A. *J. Natl. Cancer Inst*., **1990**, *82*, 749.
[9] Gong, J. G.; Costanzo, A.; Yang, H. Q.; Melino, G.; Kaelin, W. G. J.; Levrero, M.; Wang, J. Y. *Nature*, **1999**, *399*, 806.
[10] Vekris, A.; Meynard, D.; Haaz, M. C.; Bayssas, M.; Bonnet, J.; Robert, J. *Cancer Res*., **2004**, *64*, 356.
[11] Perego, P.; Giarola, M.; Righetti, S. C.; Supino, R.; Caserini, C.; Delia, D.; Pierotti, M. A.; Miyashita, T.; Reed, J. C.; Zunino, F. *Cancer Res*., **1996**, *56*, 556.
[12] Kanamori, Y.; Kigawa, J.; Minagawa, Y.; Irie, T.; Oishi, T.; Shimada, M.; Takahashi, M.; Nakamura, T.; Sato, K.; Terakawa, N. *Eur. J. Cancer*, **1998**, *34*, 1802.
[13] Pestell, K. E.; Hobbs, S. M.; Titley, J. C.; Kelland, L. R.; Walton, M. I. *Mol. Pharmacol*., **2000**, *57*, 503.
[14] Tinel, A.; Tschopp, J. *Science*, **2004**, *304*, 843.
[15] Enoksson, M.; Robertson, J. D.; Gogvadze, V.; Bu, P.; Kropotov, A.; Zhivotovsky, B.; Orrenius, S. *J. Biol. Chem*., **2004**, *279*, 49575.
[16] Guo, Y.; Srinivasula, S. M.; Druilhe, A.; Fernandes-Alnemri, T.; Alnemri, E. S. *J. Biol. Chem*., **2002**, *277*, 13430.
[17] Seth, R.; Yang, C.; Kaushal, V.; Shah, S. V.; Kaushal, G. P. *J. Biol. Chem*., **2005**, *280*, 31230.
[18] Lin, W. C.; Lin, F. T.; Nevins, J. R. *Genes Dev*., **2001**, *15*, 1833.
[19] Ianari, A.; Gallo, R.; Palma, M.; Alesse, E.; Gulino, A. J. Biol. Chem., **2004**, *279*, 30830.
[20] Wada, T.; Penninger, J. M. *Oncogene*, **2004**, *23*, 2838.
[21] Mansouri, A.; Ridgway, L. D.; Korapati, A. L.; Zhang, Q.; Tian, L.; Wang, Y.; Siddik, Z. H.; Mills, G. B.; Claret, F. X. *J. Biol. Chem*., **2003**, *278*, 19245.
[22] Sanchez-Perez, I.; Murguia, J. R.; Perona, R. *Oncogene*, **1998**, *16*, 533.
[23] Zanke, B. W.; Boudreau, K.; Rubie, E.; Winnett, E.; Tibbles, L. A.; Zon, L.; Kyriakis, J.; Liu, F. F.; Woodgett, J. R. *Curr. Biol*., **1996**, *6*, 606.
[24] Mandic, A.; Viktorsson, K.; Molin, M.; Akusjarvi, G.; Eguchi, H.; Hayashi, S. I.; Toi, M.; Hansson, J.; Linder, S.; Shoshan, M. C. *Mol. Cell Biol*., **2001**, *21*, 3684.
[25] Hayakawa, J.; Ohmichi, M.; Kurachi, H.; Ikegami, H.; Kimura, A.; Matsuoka, T.; Jikihara, H.; Mercola, D.; Murata, Y. *J. Biol. Chem*., **1999**, *274*, 31648.
[26] Potapova, O.; Haghighi, A.; Bost, F.; Liu, C.; Birrer, M. J.; Gjerset, R.; Mercola, D. *J. Biol. Chem*., **1997**, *272*, 14041.
[27] Chen, Y.-R.; Wang, X.; Templeton, D.; Davis, R. J.; Tan, T.-H. *J. Biol. Chem*., **1996**, *271*, 31929.
[28] Wei, M. C.; Zong, W. X.; Cheng, E. H.; Lindsten, T.; Panoutsakopoulou, V.; Ross, A. J.; Roth, K. A.; MacGregor, G. R.; Thompson, C. B.; Korsmeyer, S. J. *Science*, **2001**, *292*, 727.
[29] Herod, J. J.; Eliopoulos, A. G.; Warwick, J.; Niedobitek, G.; Young, L. S.; Kerr, D. *Cancer Res*., **1996**, *56*, 2178.
[30] Beale, P. J.; Rogers, P.; Boxall, F.; Sharp, S. Y.; Kelland, L. R. *Br. J. Cancer,* **2000**, *82*, 436.

[31] Zamble, D. B.; Jacks, T.; Lippard, S. J. *Proc. Natl. Acad. Sci. USA*, **1998**, *95*, 6163.
[32] Eliopoulos, A. G.; Kerr, D. J.; Herod, J.; Hodgkins, L.; Krajewski, S.; Reed, J. C.; Young, L. S. *Oncogene*, **1995**, *11*, 1217.
[33] Lock, R. B.; Stribinskiene, L. *Cancer Res*., **1996**, *56*, 4006.
[34] Yin, D. X.; Schimke, R. T. *Cancer Res*., **1995**, *55*, 4922.
[35] Brown, J. M.; Wouters, B. G. *Cancer Res*., **1999**, *59*, 1391.
[36] Wang, X.; Wong, S. C.; Pan, J.; Tsao, S. W.; Fung, K. H.; Kwong, D. L.; Sham, J. S.; Nicholls, J. M. *Cancer Res*., **1998**, *58*, 5019.
[37] Driessens, G.; Harsan, L.; Robaye, B.; Waroquier, D.; Browaeys, P.; Giannakopoulos, X.; Velu, T.; Bruyns, C. *Br. J. Cancer*, **2003**, *89*, 727.
[38] Roninson, I. B. *Cancer Res*., **2003**, *63*, 2705.
[39] d'Adda di Fagagna, F.; Reaper, P. M.; Clay-Farrace, L.; Fiegler, H.; Carr, P.; Von Zglinicki, T.; Saretzki, G.; Carter, N. P.; Jackson, S. P. *Nature*, **2003**, *426*, 194.
[40] von Zglinicki, T.; Saretzki, G.; Ladhoff, J.; d'Adda di Fagagna, F.; Jackson, S. P. *Mech. Ageing Dev*., **2005**, *126*, 111.
[41] Shay, J. W.; Roninson, I. B. *Oncogene*, **2004**, *23*, 2919.
[42] Berndtsson, M.; Hagg, M.; Panaretakis, T.; Havelka, A. M.; Shoshan, M. C.; Linder, S. *Int. J. Cancer*, **2007**, *120*, 175.
[43] Andrews, P. A.; Howell, S. B. *Cancer Cells*, **1990**, *2*, 35.
[44] Terheggen, P. M.; Emondt, J. Y.; Floot, B. G.; Dijkman, R.; Schrier, P. I.; den Engelse, L.; Begg, A. C. *Cancer Res*., **1990**, *50*, 3556.
[45] Vilpo, J. A.; Vilpo, L. M.; Szymkowski, D. E.; O'Donovan, A.; Wood, R. D. *Mol. Cell Biol*., **1995**, *15*, 290.
[46] Olaussen, K. A.; Dunant, A.; Fouret, P.; Brambilla, E.; Andre, F.; Haddad, V.; Taranchon, E.; Filipits, M.; Pirker, R.; Popper, H. H.; Stahel, R.; Sabatier, L.; Pignon, J. P.; Tursz, T.; Le Chevalier, T.; Soria, J. C. *N. Engl. J. Med*., **2006**, *355*, 983.
[47] Chang, I. Y.; Kim, M. H.; Kim, H. B.; Lee, D. Y.; Kim, S. H.; Kim, H. Y.; You, H. J. *Biochem. Biophys. Res. Commun*., **2005**, *327*, 225.
[48] Dabholkar, M.; Bostick Bruton, F.; Weber, C.; Bohr, V. A.; Egwuagu, C.; Reed, E. *J. Natl. Cancer Inst*., **1992**, *84*, 1512.
[49] Colton, S. L.; Xu, X. S.; Wang, Y. A.; Wang, G. *J. Biol. Chem*., **2006**, *281*, 27117.
[50] Yazlovitskaya, E. M.; Persons, D. L. *Anticancer Res.,* **2003**, *23*, 2275.
[51] Meng, H.; Terado, T.; Kimura, H. *Int. J. Radiat. Biol*., **1998**, *73*, 503.
[52] Zhivotovsky, B.; Kroemer, G. *Nat. Rev. Mol. Cell Biol*., **2004**, *5*, 752.
[53] Mandic, A.; Hansson, J.; Linder, S.; Shoshan, M. C. *J. Biol. Chem*., **2003**, *278*, 9100.
[54] Yang, Z.; Schumaker, L. M.; Egorin, M. J.; Zuhowski, E. G.; Guo, Z.; Cullen, K. *Clin. Cancer Res*., **2006**, *12*, 5817.
[55] Gourdier, I.; Crabbe, L.; Andreau, K.; Pau, B.; Kroemer, G. *Oncogene*, **2004**, *23*, 7449.
[56] Erdal, H.; Berndtsson, M.; Castro, J.; Brunk, U.; Shoshan, M. C.; Linder, S. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 192.
[57] Ivanov, A. I.; Christodoulou, J.; Parkinson, J. A.; Barnham, K. J.; Tucker, A.; Woodrow, J.; Sadler, P. J. *J. Biol. Chem*., **1998**, *273*, 14721.
[58] Hagrman, D.; Goodisman, J.; Dabrowiak, J. C.; Souid, A. K. *Drug Metab. Dispos*., **2003**, *31*, 916.
[59] Faivre, S.; Chan, D.; Salinas, R.; Woynarowska, B.; Woynarowski, J. M. *Biochem. Pharmacol*., **2003**, *66*, 225.
[60] Speelmans, G.; Staffhorst, R. W.; Versluis, K.; Reedijk, J.; de Kruijff, B. *Biochemistry*, **1997**, *36*, 10545.
[61] Mandic, A.; Viktorsson, K.; Strandberg, L.; Heiden, T.; Hansson, J.; Linder, S.; Shoshan, M. C. *Mol. Cell Biol*., **2002**, *22*, 3003.
[62] Nawrocki, S. T.; Carew, J. S.; Pino, M. S.; Highshaw, R. A.; Dunner, K., Jr.; Huang, P.; Abbruzzese, J. L.; McConkey, D. *Cancer Res.,* **2005**, *65*, 11658.
[63] Micheau, O.; Solary, E.; Hammann, A.; Dimanche-Boitrel, M. T. *J. Biol. Chem*., **1999**, *274*, 7987.
[64] Lacour, S.; Hammann, A.; Grazide, S.; Lagadic-Gossmann, D.; Athias, A.; Sergent, O.; Laurent, G.; Gambert, P.; Solary, E.; Dimanche-Boitrel, M. T. *Cancer, Res*., **2004**, *64*, 3593.
[65] Preston, T. J.; Abadi, A.; Wilson, L.; Singh, G. *Adv. Drug Deliv. Rev*., **2001**, *49*, 45.
[66] Park, S. Y.; Chang, I.; Kim, J. Y.; Kang, S. W.; Park, S. H.; Singh, K.; Lee, M. S. *J. Biol. Chem*., **2004**, *279*, 7512.
[67] Driessens, G.; Harsan, L.; Browaeys, P.; Giannakopoulos, X.; Velu, T.; Bruyns, C. *Ann. N. Y. Acad. Sci*., **2003**, *1010*, 775.
[68] Eom, Y. W.; Kim, M. A.; Park, S. S.; Goo, M. J.; Kwon, H. J.; Sohn, S.; Kim, W. H.; Yoon, G.; Choi, K. S. *Oncogene*, **2005**, *24*, 4765.
[69] Banfi, B.; Malgrange, B.; Knisz, J.; Steger, K.; Dubois-Dauphin, M.; Krause, K. H. *J. Biol. Chem*., **2004**, *279*, 46065.

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