Cancer Chemother Pharmacol (2009) 64:607–614
DOI 10.1007/s00280-008-0912-6

ORIGINAL ARTICLE

RAIDD expression is impaired in multidrug resistant osteosarcoma cell lines

Cao Yang · Francis J. Hornicek · Kirkham B. Wood · Joseph H. Schwab · Henry Mankin · Zhenfeng Duan

Received: 5 September 2008 / Accepted: 15 December 2008 / Published online: 6 January 2009
© Springer-Verlag 2008

Abstract
Purpose To identify the apoptosis genes involved in the multidrug resistant phenotype of osteosarcoma.
Methods Multidrug resistant human osteosarcoma cell line (U-2 OS MR) and a drug sensitive parental cell line (U-2 OS) were both treated with paclitaxel and analyzed by the gene array containing 96 apoptosis associated genes. The different expression of the special apoptosis associated genes were further analyzed by Western blot in the multidrug resistant osteosarcoma cell lines (U-2 OS MR, KH OS R2) and the drug sensitive parental cell lines (U-2 OS, KH OS). One of the disregulated gene, RAIDD, was transfected into the multidrug resistant osteosarcoma cells for functional studies.
Results RAIDD showed signs of significant expression in the U-2 OS cells after being treated with paclitaxel ($P < 0.01$). However, the induction of RAIDD did not occur in U-2 OS MR cells ($P = 0.2$). Subsequent analysis by Western blot confirmed the deficiency of the expression of RAIDD protein in U-2 OS MR. On the contrary, the expression of RAIDD could be significantly induced by paclitaxel and doxorubicin in U-2 OS cells as both time and dosage were deciding factors. It also demonstrated the cleavage of PARP associated with RAIDD expression in U-2 OS cells, but not however in U-2 OS MR cells after being treated with paclitaxel or doxorubicin. Similar results were found in osteosarcoma multidrug resistant cell line KH OS R2 and the drug sensitive parental cell line KH OS. Furthermore, over-expression of RAIDD in multidrug resistant cell lines could possibly reverse drug resistant phenotypes.
Conclusion This study indicate that impaired expression of RAIDD in drug induced apoptosis may play a role in the multidrug resistance of osteosarcoma cells.

Keywords RAIDD · Multidrug resistance · Osteosarcoma · Apoptosis

C. Yang · F. J. Hornicek · K. B. Wood · J. H. Schwab · H. Mankin · Z. Duan (✉)
Department of Orthopaedic Surgery,
Massachusetts General Hospital, Boston, MA 02114, USA
e-mail: zduan@partners.org

C. Yang · F. J. Hornicek · J. H. Schwab · H. Mankin · Z. Duan
Sarcoma Biology Laboratory,
Center for Sarcoma and Connective Tissue Oncology,
Massachusetts General Hospital, Boston, MA 02114, USA

C. Yang
Department of Orthopaedic Surgery, Union Hospital,
Tongji Medical College, Huazhong University of Science
and Technology, 430022 Wuhan, China

Introduction

Osteosarcoma is the most frequent highly malignant tumor of bone in children and adolescents and exhibits a peak during the second and third decade of life [22]. Chemotherapy has significantly improved the cure rate of patients with localized osteosarcoma from 15 to 20% achieved with surgery alone to approximately 70% [14, 21, 22]. Nonetheless, nearly one-third of osteosarcoma patients experience recurrent or progressive disease, most of which are due to the development of resistance by the osteosarcoma cell to chemotherapeutic drugs [22].

A number of mechanisms have been proposed for the cancer cells to develop intrinsic or acquired resistance to one or more chemotherapeutic agents. Depending on the agent and the cellular context, these include drug inactivation, mutation of the drug target, up-regulation and

down-regulation of the drug target, decreased drug uptake, increased drug elimination and increased DNA damage repair [3, 15]. There are many agents which were proven to be effective on osteosarcoma, from conventional cytotoxic agents to innovative tyrosine kinase inhibitors. However, resistance to these agents is still a challenge in the treatment of osteosarcoma patients [3]. It is necessary to improve the understanding of the molecular basis of chemotherapeutic drug resistance for the development of new drugs and therapeutic regimens for a more effective treatment of aggressive and recurrent multidrug resistant osteosarcoma.

Apoptosis has been accepted as a fundamental component in the pathogenesis of cancer. The origin of cancer involves deregulated cellular proliferation and the suppression of apoptotic processes, ultimately leading to tumor establishment and growth [8]. Chemotherapeutic drugs of differing structure and specificity induce the characteristic morphological changes associated with apoptosis, and it is now believed that apoptotic pathways contribute to the cytotoxic action of most chemotherapeutic drugs [7, 9, 12]. There is growing evidence that disregulation of apoptotic pathways contribute to drug resistance [9, 20]. Studies on how apoptotic pathways are deregulated in drug-resistant cancer cell is therefore critical both to the understanding of the mechanism of drug-resistance and for the development of new, more effective drugs.

To further identify the deregulated apoptotic genes in multidrug resistant osteosarcoma cell lines, we analyzed the apoptosis-associated genes by using human apoptosis gene array. We provide evidence for the first time that an impairment of RAIDD (RIP associated ICH-1/CED-3-homologous protein with a death domain) induction is associated with multidrug resistance or apoptosis resistance in osteosarcoma cells.

Materials and methods

Cell culture

The human osteosarcoma cell line U-2 OS were obtained from the American Type Tissue Collection (Rockville, MD). The Multidrug resistant cell line U-2 OS MR was established in our laboratory and was resistant to paclitaxel, doxorubicin, mitoxantrone and vincristine as compared to sensitive parental cell line U-2 OS. The human osteosarcoma cell line KH OS and the multidrug resistant cell line KH OS R2 were kindly provided by Dr. Efstathios S. Gonos (National Hellenic Research Foundation, Athens, Greece) [11]. The cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin (all obtained from invitrogen, Carlsbad, CA).

RNA isolation and human apoptosis gene array

Total RNA of the cells treated with paclitaxel and the control cells was extracted using Trizol reagent (Invitrogen, Carlsbad, CA) and quantified by the spectrophotometer (Beckman DU-640, Beckman Instruments, Inc., Columbia, MD). RNA quality was determined via ethidium bromide staining post agarose/formaldehyde gel electrophoresis. A prefabricated gene membrane array was used to evaluate the expression of 96 genes involved in apoptosis (Human Apoptosis Q Series Array, SuperArray Bioscience Corp., Frederick, MD). This array includes the TNF ligands and TNF receptors; members of the bcl-2, caspase, IAP, TRAF, CARD, death domain, death effecter domain, and CIDE families; as well as genes involved in the p53 and ATM pathways. The isolated RNA underwent reverse transcription with reverse transcriptase and oligodT mixture. The cDNA was then labeled with ^{32}P-dCTP by Linear Polymerase Reaction reagents (SuperArray Bioscience Corp.), and the radiolabeled cDNA probe was used in the gene expression array. The membranes were pre-hybridized with sheared salmon sperm DNA (Sigma, St Louis, MO) and subsequent hybridization with the radiolabeled cDNA probe overnight. The membranes were then washed once with 2% SSC solution and washed twice with 0.1% SSC solution to decrease background noise, and was then exposed to x-ray film. Developed films were then scanned with an Epson Perfection 4490 Photo Scanner. The image data extraction and data analysis were performed using the web-based GEArray Analyzer (SuperArray Bioscience). All raw signal intensities were corrected for background by subtracting the signal intensity of three blanks and three pUC18 DNA. All signal intensities were also normalized to that of the housekeeping genes, including β-actin, GAPDH, cyclophilin A and ribosomal protein L13a. These corrected, normalized signals were then used to estimate the relative abundance of particular gene transcripts.

Western blot analysis

Protein lysates from cells were generated through lysis with 1 × RIPA Lysis Buffer (Upstate Biotechnology, Charlottesville, VA). The concentration of the protein was determined by Protein Assay Reagents (Bio-Rad, Hercules, CA) and spectrophotometer (Beckman DU-640, Beckman Instruments, Inc., Columbia, MD). Forty micrograms of total protein was processed on Nu-Page 4–12% Bis-Tris Gel (Invitrogen) and transferred to a pure nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA). Antibodies directed against RAIDD were obtained from Sigma (Sigma, St Louis, MO). Antibodies directed against PARP were obtained from Cell Signaling(Cell Signaling, Danvers, MA). Antibodies directed against actin were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Primary

antibodies were incubated at 1:1,000 dilution in Tris-buffered saline, pH 7.4, with 0.1% Tween 20 and overnight at 4°C. Signal was generated through incubation with horseradish peroxidase-conjugated secondary antibodies (Bio-Rad, Hercules, CA) incubated in Tris-buffered saline, pH 7.4, with 5% nonfat milk and 0.1% Tween 20 at 1:2,000 dilution for 1 h at room temperature. Positive immunoreactions were detected by using SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL).

pIRES$_{RAIDD}$ expression vector construction

Clonetech's (Palo Alto, CA) mammalian expression vector pIRESneo contains the IRES of the encephalomyocarditis virus, which permits the translation of two ORFs from one mRNA. The expression cassette of pIRESneo contains the human CMV major immediate early promoter/enhancer (pCMV) followed by a multiple cloning site and synthetic intron known to enhance the stability of the mRNA. A 950-bp cDNA fragment containing the full ORF of human RAIDD was amplified by RT-PCR from the RNA of U-2 OS. RT-PCR primers were forward 5′-ATAAGAAT*GCGGCCGC*GAGAAATGGAGGCCAGAGA-3′ to introduce a *Not*I site as underlined and reverse 5′ GGT*GGATCC*TGGAGGCACCATCACTCCAACATG-3′ to introduce a *Bam*HI site as underlined. The resulting RAIDD RT-PCR product was cloned to pCR 2.1 vector using Invitrogen's Original TA Cloning Kit. After sequence confirmation, RAIDD was cut from the pCR 2.1 vector, purified, sub-cloned to the multiple cloning site of expression vector pIRESneo, and subsequently sequenced to confirm the correct ORF. Expression of RAIDD cDNA was under the control of the pCMV.

Gene transfection

Gene transfections were performed using Lipofect Amine Plus reagents (Life Technologies, Inc.) as follows: 5×10^5 U-2 OS MR cells were plated into 90-mm tissue culture dishes and cultured overnight. Before transfection, the growth medium was replaced with serum-free RPMI 1640 and cultured for 3 h. Lipofect Amine reagent, containing 5 μg of pIRESempty or pIRES$_{RAIDD,}$ was combined with Plus reagent and applied to the cells. After culture for 4 h, the media were replaced with RPMI 1640 containing 10% fetal bovine serum. The expression of RAIDD in the transfected cells was examined by Western blot.

Cytotoxicity assay

The In vitro cytotoxicity assays were performed by MTT assay as previously described [5]. MTT was obtained from Sigma (St Louis, MO). Briefly, 2×10^3 cells per well were plated in 96-well plates. After culture for 7 days, 50 μl of MTT (5 mg/ml PBS) was added to each well and incubated for 4 h. After dissolving the resulting formazan product with acid isopropanol, the absorbance (A490) was read on a BT 2000 Microkinetics Reader (Bio-Tek Instrument, Inc., Winooski, VT) at a wavelength of 490 nm. Experiments were performed in duplicate.

Data analysis

Values show are representative of triplicate determinations in two or more experiments. Treatment effects were evaluated using a two-sided Student's t test (GraphPad PRISM® 4 software, GraphPad Software, San Diego, CA). Errors are SD of averaged results and $P < 0.05$ values were accepted as a significant difference between means.

Results

RAIDD gene was upregulated in U-2 OS cells but not in U-2 OS MR cells after treated with paclitaxel

We compared the expression of 96 apoptosis associated genes between drug sensitive U-2 OS cells and drug resistant U-2 OS MR cells. After treatment with 0.01 μM paclitaxel for 24 h, the expression of RAIDD in the drug treated U-2 OS cells was upregulated significantly compared with untreated control cells ($P < 0.01$). On the contrary, the expression of RAIDD in the U-2 OS MR cells was not upregulated significantly as compared with untreated control cells ($P = 0.2$) (Fig. 1). The expression of p53, Bcl-2

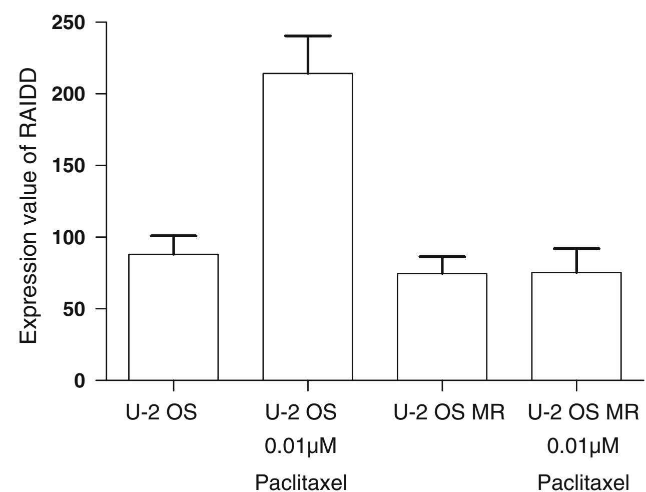

Fig. 1 The expression of 96 apoptosis associated genes in the U-2 OS and U-2 OS MR cells after treatment with or without paclitaxel were analyzed by gene array. After treatment with 0.01 μM paclitaxel, the expression of RAIDD in the U-2 OS cells was upregulated significantly compared with untreated control cells. On the contrary, the expression of RAIDD in the U-2 OS MR cells was not upregulated significantly as compared with untreated control cells

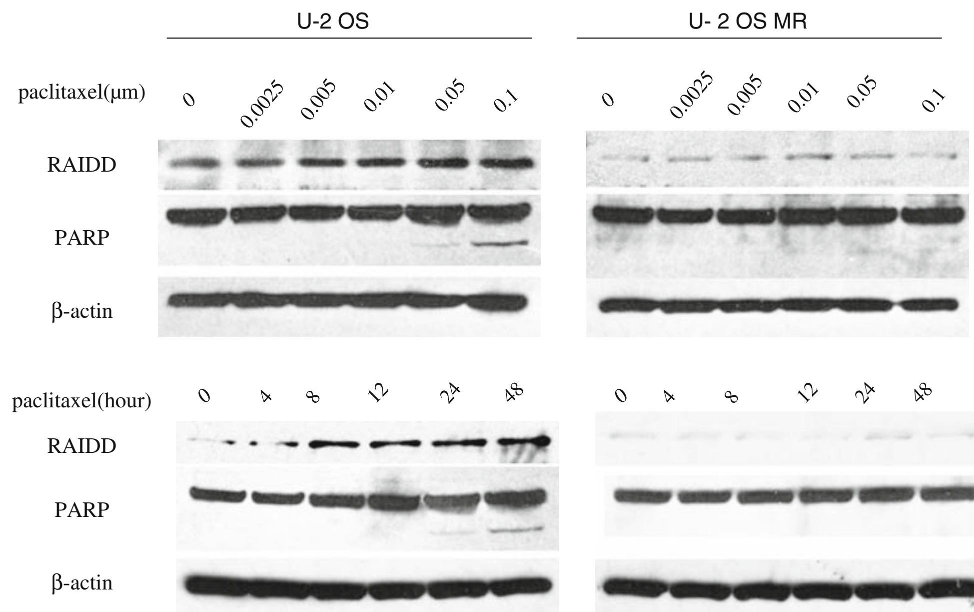

Fig. 2 After treatment with paclitaxel, dose- and time-dependent induction of RAIDD was demonstrated in U-2 OS cells, but not in U-2 OS MR cells. The cleavage of PARP was also found in U-2 OS cells but not in U-2 OS MR cells after treatment with paclitaxel

family such as Bcl-2, Bax and Bcl-$X_{L,}$ TNF-related apoptosis-induced ligand (TRAIL) and its receptors DcR1, DcR2, DR4 and DR5, IAP family such as survivin, Caspase family had no difference between multidrug resistant U-2 OS MR cells and the parental U-2 OS cells.

RAIDD protein was upregulated in drug sensitive osteosarcoma cells but not in multidrug resistant cells

After treating the cells with paclitaxel in various concentration for 24 h or with 0.01 μM paclitaxel for 0–48 h, Western blot analysis demonstrated both dose- and time-dependent induction of RAIDD by paclitaxel in U-2 OS cells. In contrast, the dose- and time-dependent induction of RAIDD protein expression by paclitaxel was not found in U-2 OS MR cells. It also demonstrated the cleavage of PARP in U-2 OS cells after treatment with paclitaxel. On the contrary, the cleavage of PARP was not found in U-2 OS MR cells after treatment with paclitaxel (Fig. 2).

As U-2 OS MR cells had also acquired enhanced cross-resistance to various chemotherapeutic drugs, we analyzed whether the expression of RAIDD could be upregulated after treatment with various chemotherapeutic drugs. After treating the cells with doxorubicin in various concentration for 24 h or with 0.5 μM doxorubicin for 0–48 h, Western blot analysis also demonstrated both dose- and time-dependent induction of RAIDD by doxorubicin in U-2 OS cells but not in multidrug resistant U-2 OS MR cells. The cleavage of PARP was also found in U-2 OS cells but not in U-2 OS MR cells after treatment with doxorubicin (Fig. 3).

In addition, to further evaluate whether the expression of RAIDD was deregulated in other multidrug resistant osteosarcoma cell lines, the KH OS R2 cells and the sensitive parental cells KH OS were treated with 0.01 μM paclitaxel or 0.5 μM doxorubicin for 0–48 h. The cells were also treated with paclitaxel or doxorubicin in various concentrations for 24 h. Like U-2 OS, Western blot analysis demonstrated the dose- and time-dependent induction of RAIDD by paclitaxel in KH OS cells. On the other hand, the dose- and time-dependent induction of RAIDD by paclitaxel was not found in KH OS R2 cells. The cleavage of PARP was also found in U-2 OS cells but not in U-2 OS MR cells after treatment with paclitaxel. Western blot analysis also demonstrated the dose- and time-dependent induction of RAIDD by doxorubicin in KH OS cells but not in multidrug resistant KH OS R2 cells. The cleavage of PARP was also found in KH OS cells but not in KH OS R2 cells after being treated with doxorubicin (Fig. 4).

RAIDD could reverse the drug resistance of multidrug resistant osteosarcoma cells

The U-2 OS MR and KH OS R2 cells were transfected with pIRESempty or $pIRES_{RAIDD}$. Western blot analysis demonstrated high level expression of RAIDD in the U-2 OS MR and KH OS R2 cells transfected with $pIRES_{RAIDD}$ (Fig. 5). To evaluate if the RAIDD could reverse the drug resistance of the multidrug resistant cells, the U-2 OS MR cells and KH OS R2 cells transfected with $pIRES_{RAIDD}$ were treated with paclitaxel or doxorubicin. The naïve U-2 OS MR and

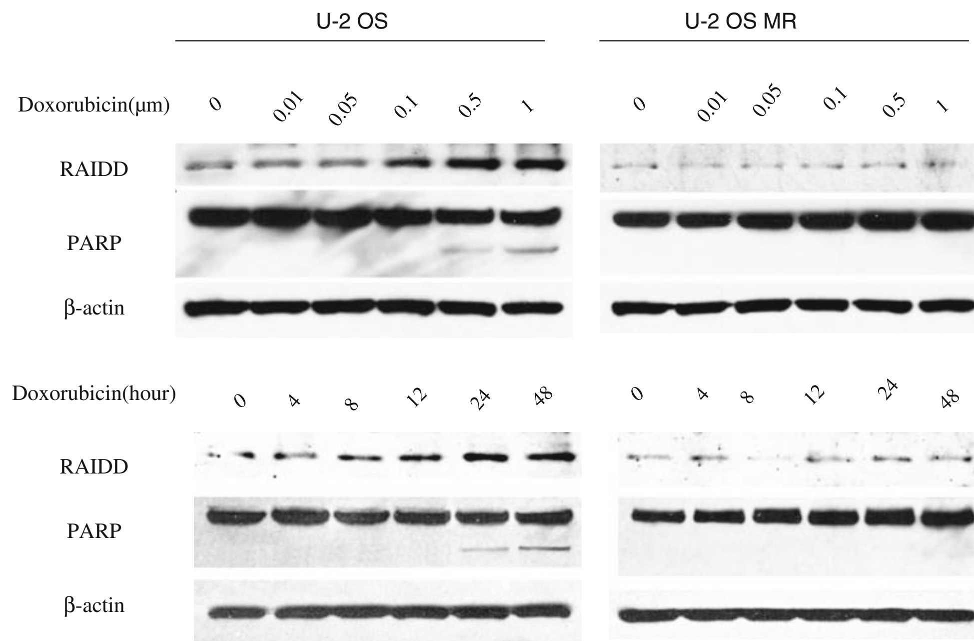

Fig. 3 After treatment with doxorubicin, dose- and time-dependent induction of RAIDD was demonstrated in U-2 OS cells, but not in U-2 OS MR cells. The cleavage of PARP was also found in U-2 OS cells but not in U-2 OS MR cells after treatment with doxorubicin

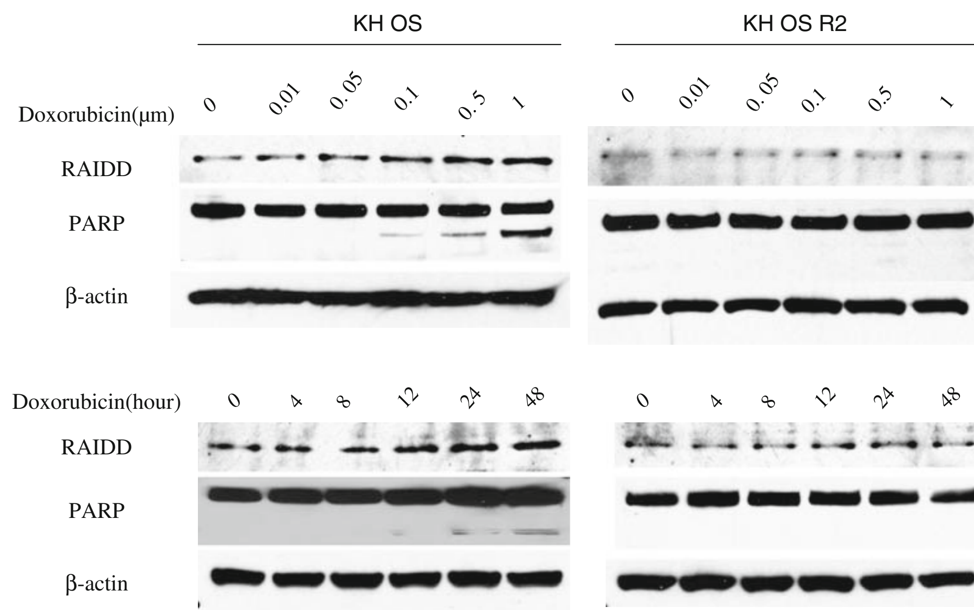

Fig. 4 Western blot analysis demonstrated the dose- and time-dependent induction of RAIDD by doxorubicin in KH OS cells, but not in KH OS R2 cells. The cleavage of PARP was also found in KH OS cells but not in KH OS R2 cells after treatment with doxorubicin

KH OS R2 cells were also treated with paclitaxel or doxorubicin as a control. The cells were incubated for 7 days. The MTT assay demonstrated that the multidrug resistant cells U-2 OS MR and KH OS R2 which were transfected with RAIDD become more sensitive to paclitaxel and doxorubicin than the naïve multidrug resistant cells (Fig. 6). The IC50 values for doxorubicin in parental U-2 OS MR cells (IC50 = 0.307) is 5.5 fold than transfected U-2 OS MR cells (IC50 = 0.056) and the IC50 values for paclitaxel in parental U-2 OS MR cells (IC50 = 0.021) is 7.8 fold than transfected U-2 OS MR cells (IC50 = 0.0027). The IC50 values for doxorubicin in parental KH OS R2 cells (IC50 = 2.43) is 5 fold than transfected KH OS R2 cells (IC50 = 0.38) and the IC50 values for paclitaxel in parental KH OS R2 cells (IC50 = 0.2) is 4 fold than transfected KH OS R2 cells (IC50 = 0.05). Western blot

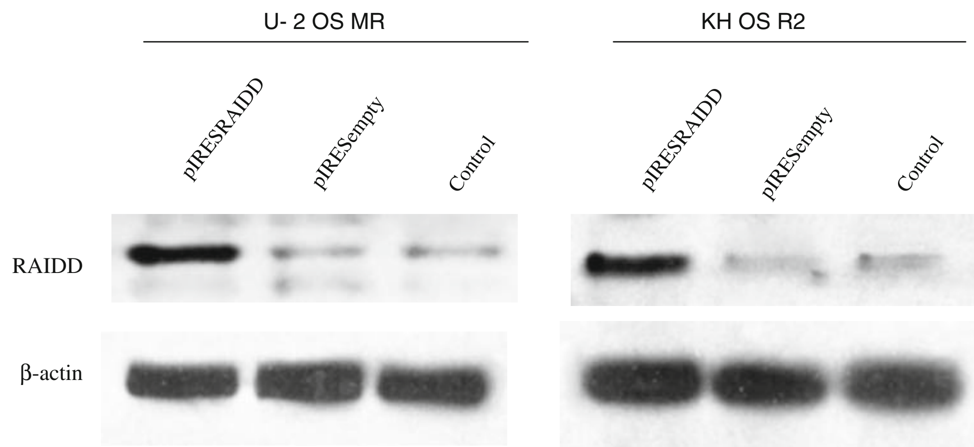

Fig. 5 Western blot demonstrated high level expression of RAIDD in the U-2 OS MR and KH OS R2 cells transfected with $pIRES_{RAIDD}$

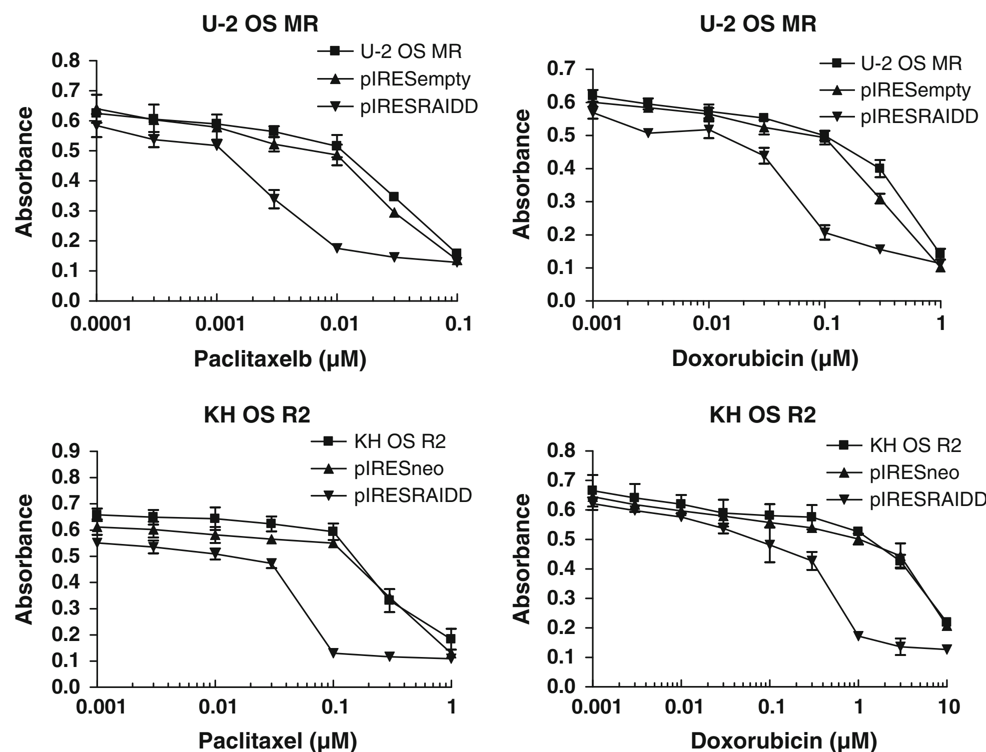

Fig. 6 The MTT assay demonstrated that the multidrug resistant cells U-2 OS MR and KH OS R2, which transfected with RAIDD, become more sensitive to paclitaxel and doxorubicin than the naïve multidrug resistant cells

demonstrate the apoptosis of U-2 OS MR and KH OS R2 cells transfected with RAIDD after treatment with paclitaxel and doxorubicin (Fig. 7).

Discussion

Apoptosis regulation is controlled through a complex interplay of pro- and anti-apoptotic signals that in turn set an apoptotic threshold [8]. Drug sensitive cancer cells usually have a low apoptotic threshold and thus an inclination toward therapy-induced apoptosis, while multidrug resistant cells exhibit resistance to apoptosis through increased expression of anti-apoptotic genes or decreased expression of pro-apoptotic genes [9, 13]. Thus, the variable expression and complex interaction of multiple factors involved in the regulation of the apoptotic responses may represent critical events underlying the sensitivity/resistance status of tumour cells [18]. Nevertheless, the role of apoptosis genes in acquiring the multidrug resistant phenotype in osteosarcoma is still unclear. In this study, the expression of 96 genes associated with apoptosis was compared between the

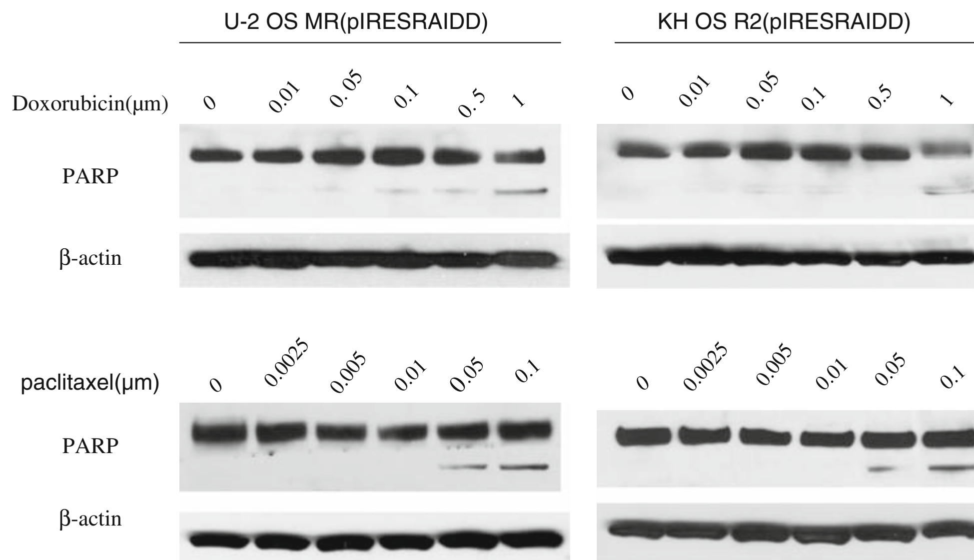

Fig. 7 Western blot demonstrate the cleavage of PARP in U-2 OS MR and KH OS R2 cells transfected with RAIDD after treatment of paclitaxel and doxorubicin

multidrug resistant cell line U-2 OS MR and the sensitive cell line U-2 OS. In the 96 apoptosis associated genes comparison, RAIDD mRNA was differently expressed in the multidrug resistant cell line and the sensitive cell line. The expression of RAIDD could be upregulated by paclitaxel in the sensitive osteosarcoma cell line. The induction of RAIDD mRNA by paclitaxel in sensitive cell line was accompanied by an increase in RAIDD protein. The expression of RAIDD could also be induced by doxorubicin in the sensitive cell line. The expression of RAIDD in the sensitive osteosarcoma cells was accompanied with the cleavage of PARP after being treated with paclitaxel or doxorubicin. This suggests that RAIDD may play a role in drug-induced apoptosis in osteosarcoma cell. On the other hand, RAIDD couldn't be induced in multidrug resistant osteosarcoma cell line after treatment with paclitaxel and doxorubicin. The deficiency in expression of RAIDD in drug resistant osteosarcoma cells was also accompanied with the deficiency in cleavage of PARP after treatment with paclitaxel or doxorubicin. The different expression of RAIDD accompanied with cleavage PARP was also confirmed in the other multidrug resistant cell line KH OS R2 and its parental cell line KH OS. Therefore, these studies indicate that a deficiency in RAIDD induced apoptosis may play a role in the multidrug resistance of osteosarcoma cell.

RAIDD belongs to the proteins of death domain (DD) superfamily which mediate assembly of oligomeric signaling complexes for the activation of caspases and kinases [1, 4]. RAIDD contains both a N-terminal caspase recruitment domain (CARD) and a C-terminal death domain (DD), and acts as a bridge between caspase-2 and p53-induced protein with death domain (PIDD) [23, 25]. While RAIDD and PIDD interact with each other via their DDs, RAIDD and caspase-2 interact with each other via their CARDs. RAIDD, PIDD, and caspase-2 spontaneously assemble into a high-molecular-weight complex, the PIDDosome, which is implicated in activation of caspase-2 in response to genotoxic stress [16, 17]. The overexpression of RAIDD or PIDD results in spontaneous activation of caspase-2 and sensitization to apoptosis by genotoxic stimuli. Because PIDD functions in p53-mediated apoptosis, the PIDDosome is likely to regulate apoptosis induced by genotoxins. However, apoptosis caused by PIDD was dependent on the presence of endogenous RAIDD, because ablation of RAIDD expression by small interfering RNA (siRNA) blocked caspase-2 processing [2, 19, 25, 27]. Cells lacking RAIDD failed to activate caspase-2 after heat shock treatment and showed resistance to apoptosis [26]. Although the precise mechanisms of drug induced apoptosis in osteosarcoma remains unclear, our results indicate that the expression of RAIDD may play an important role in this apoptotic pathway. In the multidrug resistant cell lines, the deficiency of the expression of RAIDD may be the reason of the deficiency of drug induced apoptosis, which result in multidrug resistant characteristics of the osteosarcoma cells. When RAIDD was expressed in the multidrug resistant cells, it reverted the drug resistance of the cells. It was further confirmed that the deficiency of the expression of RAIDD may play a role in multidrug resistance of osteosarcoma.

Previous studies demonstrated that p53, IAP family such as survivin, Bcl-2 family such as Bax, TRAIL and its receptors DR4, DcR1 and DcR2 may play a role in drug resistance in sarcoma cell lines [6, 10, 24, 28]. However, the gene array analysis demonstrated that the expression of these genes had no difference between multidrug resistant U-2 OS MR cells and the parental U-2 OS cells. All of

these genes could not be induced by paclitaxel, both in the U-2 OS MR cells and U-2 OS cells.

In conclusion, we have demonstrated that the pro- or anti-apoptotic regulatory mechanisms may be associated with multidrug resistance of osteosarcoma cells. The deficiency of RAIDD induced apoptosis may play a role in multidrug resistance in osteosarcoma cell. Furthermore, it also suggests that RAIDD may serve as a potential target for overcoming drug resistance in osteosarcoma.

Acknowledgments Dr. C. Yang is supported through a grant from the Chinese Council of Scholarship. Dr. Z. Duan is supported, in part, through a grant from the National Cancer Institute, NIH (Nanotechnology Platform Partnership), R01-CA119617. Support has also been provided by the Gaetagno and Wechsler funds.

References

1. Ahmad M, Srinivasula SM, Wang L, Talanian RV, Litwack G, Fernandes-Alnemri T, Alnemri ES (1997) CRADD, a novel human apoptotic adaptor molecule for caspase-2, and FasL/tumor necrosis factor receptor-interacting protein RIP. Cancer Res 57:615–619
2. Berube C, Boucher LM, Ma W, Wakeham A, Salmena L, Hakem R, Yeh WC, Mak TW, Benchimol S (2005) Apoptosis caused by p53-induced protein with death domain (PIDD) depends on the death adapter protein RAIDD. Proc Natl Acad Sci USA 102:14314–14320
3. Chou AJ, Gorlick R (2006) Chemotherapy resistance in osteosarcoma: current challenges and future directions. Expert Rev Anticancer Ther 6:1075–1085
4. Duan H, Dixit VM (1997) RAIDD is a new ‘death’ adaptor molecule. Nature 385:86–89
5. Duan Z, Duan Y, Lamendola DE, Yusuf RZ, Naeem R, Penson RT, Seiden MV (2003) Overexpression of MAGE/GAGE genes in paclitaxel/doxorubicin-resistant human cancer cell lines. Clin Cancer Res 9:2778–2785
6. Fayette J, Blay JY (2005) Genetic predictors for drug resistance in soft tissue sarcoma: a review of publications in 2004. Curr Opin Oncol 17:370–375
7. Hannun YA (1997) Apoptosis and the dilemma of cancer chemotherapy. Blood 89:1845–1853
8. Hunter AM, LaCasse EC, Korneluk RG (2007) The inhibitors of apoptosis (IAPs) as cancer targets. Apoptosis 12:1543–1568
9. Johnstone RW, Ruefli AA, Lowe SW (2002) Apoptosis: a link between cancer genetics and chemotherapy. Cell 108:153–164
10. Liu F, Xie ZH, Cai GP, Jiang YY (2007) The effect of survivin on multidrug resistance mediated by P-glycoprotein in MCF-7 and its adriamycin resistant cells. Biol Pharm Bull 30:2279–2283
11. Lourda M, Trougakos IP, Gonos ES (2007) Development of resistance to chemotherapeutic drugs in human osteosarcoma cell lines largely depends on up-regulation of Clusterin/Apolipoprotein J. Int J Cancer 120:611–622
12. Lowe SW, Lin AW (2000) Apoptosis in cancer. Carcinogenesis 21:485–495
13. Makin G, Hickman JA (2000) Apoptosis and cancer chemotherapy. Cell Tissue Res 301:143–152
14. Marcove RC, Mike V, Hajek JV, Levin AG, Hutter RV (1970) Osteogenic sarcoma under the age of twenty-one. A review of one hundred and forty-five operative cases. J Bone Joint Surg Am 52:411–423
15. Mimeault M, Hauke R, Batra SK (2007) Recent Advances on the Molecular Mechanisms Involved in the Drug Resistance of Cancer Cells and Novel Targeting Therapies. Clin Pharmacol Ther 83:673–691
16. Park HH, Logette E, Raunser S, Cuenin S, Walz T, Tschopp J, Wu H (2007) Death domain assembly mechanism revealed by crystal structure of the oligomeric PIDDosome core complex. Cell 128:533–546
17. Park HH, Wu H (2006) Crystal structure of RAIDD death domain implicates potential mechanism of PIDDosome assembly. J Mol Biol 357:358–364
18. Perego P, Righetti SC, Supino R, Delia D, Caserini C, Carenini N, Bedogne B, Broome E, Krajewski S, Reed JC, Zunino F (1997) Role of apoptosis and apoptosis-related proteins in the cisplatin-resistant phenotype of human tumor cell lines. Apoptosis 2:540–548
19. Pick R, Badura S, Bosser S, Zornig M (2006) Upon intracellular processing, the C-terminal death domain-containing fragment of the p53-inducible PIDD/LRDD protein translocates to the nucleoli and interacts with nucleolin. Biochem Biophys Res Commun 349:1329–1338
20. Prabhudesai SG, Rekhraj S, Roberts G, Darzi AW, Ziprin P (2007) Apoptosis and chemo-resistance in colorectal cancer. J Surg Oncol 96:77–88
21. Provisor AJ, Ettinger LJ, Nachman JB, Krailo MD, Makley JT, Yunis EJ, Huvos AG, Betcher DL, Baum ES, Kisker CT, Miser JS (1997) Treatment of nonmetastatic osteosarcoma of the extremity with preoperative and postoperative chemotherapy: a report from the Children’s Cancer Group. J Clin Oncol 15:76–84
22. Schwartz CL, Gorlick R, Teot L, Krailo M, Chen Z, Goorin A, Grier HE, Bernstein ML, Meyers P (2007) Multiple drug resistance in osteogenic sarcoma: INT0133 from the Children’s Oncology Group. J Clin Oncol 25:2057–2062
23. Shearwin-Whyatt LM, Harvey NL, Kumar S (2000) Subcellular localization and CARD-dependent oligomerization of the death adaptor RAIDD. Cell Death Differ 7:155–165
24. Sturm I, Petrowsky H, Volz R, Lorenz M, Radetzki S, Hillebrand T, Wolff G, Hauptmann S, Dorken B, Daniel PT (2001) Analysis of p53/BAX/p16(ink4a/CDKN2) in esophageal squamous cell carcinoma: high BAX and p16(ink4a/CDKN2) identifies patients with good prognosis. J Clin Oncol 19:2272–2281
25. Tinel A, Tschopp J (2004) The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 304:843–846
26. Tu S, McStay GP, Boucher LM, Mak T, Beere HM, Green DR (2006) In situ trapping of activated initiator caspases reveals a role for caspase-2 in heat shock-induced apoptosis. Nat Cell Biol 8:72–77
27. Vakifahmetoglu H, Olsson M, Orrenius S, Zhivotovsky B (2006) Functional connection between p53 and caspase-2 is essential for apoptosis induced by DNA damage. Oncogene 25:5683–5692
28. Violette S, Poulain L, Dussaulx E, Pepin D, Faussat AM, Chambaz J, Lacorte JM, Staedel C, Lesuffleur T (2002) Resistance of colon cancer cells to long-term 5-fluorouracil exposure is correlated to the relative level of Bcl-2 and Bcl-X(L) in addition to Bax and p53 status. Int J Cancer 98:498–504