Inhibition of Survivin Is Associated with Zoledronic Acid-induced Apoptosis of Prostate Cancer Cells

Article *in* Anticancer research · March 2016

11 authors, including:

Claudio Simeone
Università degli Studi di Brescia
198 PUBLICATIONS **1,055** CITATIONS
SEE PROFILE

Maurizio Memo
Università degli Studi di Brescia
291 PUBLICATIONS **6,621** CITATIONS
SEE PROFILE

Sandra Sigala
Università degli Studi di Brescia
83 PUBLICATIONS **1,051** CITATIONS
SEE PROFILE

Titolo rivista/libro: Anticancer research

Titolo articolo/sezione: Inhibition of Survivin Is Associated with Zoledronic Acid-induced Apoptosis ofProstate Cancer Cells

Autore/i: Fragni M, Bonini SA, Stabile A, Bodei S, Cristinelli L, Simeone C, Zani D, Spano PF, Berruti A, Memo M, Sigal

ISSN: 1791-7530

DOI:

Anno: 2016

Volume: 36

Fascicolo: 3

Editore:

Pag. iniziale: 913

Pag. finale: 920

Inhibition of Survivin Is Associated with Zoledronic Acid-induced Apoptosis of Prostate Cancer Cells

MARTINA FRAGNI[1], SARA ANNA BONINI[1], ARMANDO STABILE[2], SERENA BODEI[1], LUCA CRISTINELLI[3], CLAUDIO SIMEONE[3], DANILO ZANI[3,5], PIER FRANCO SPANO[1], ALFREDO BERRUTI[4], MAURIZIO MEMO[1] and SANDRA SIGALA[1]

[1]*Section of Pharmacology, Department of Molecular and Translational Medicine,*
[3]*Urology Unit University of Brescia, Brescia, Italy;*
[4]*Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Brescia, Italy;*
[2]*Unit of Urology, Scientific Institute and University San Raffaele Hospital, Milan, Italy;*
[5]*Division of Urology, City of Brescia Clinical Institute, Brescia, Italy*

Abstract. *Background/Aim: Evidence suggests that zoledronic acid (ZA) exerts direct antitumor effects on cancer cells but the underlying mechanisms of these actions are unknown. This study investigated the possible involvement of survivin in the antiproliferative effects of ZA in prostate cancer. Materials and Methods: 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye reduction assay was used to assess cell viability and acridine orange/ethidium bromide double staining to analyze cell death. Human Apoptosis Array evaluated the expression of apoptosis-related proteins. Survivin protein was measured by western blot technique and miR-203 levels were quantified by quantitative real-time polymerase chain reaction. Results: ZA induced inhibition of cell proliferation and apoptosis activation, with down-regulation of survivin protein. A negative regulation at gene expression level may be hypothesized because we observed a significant decrease of survivin mRNA level and an increase of miR-203 expression after ZA exposure. Conclusion: This study provides evidence that ZA may directly inhibit cancer cell proliferation, identifying survivin as one of its downstream targets.*

Prostate cancer (PCa) is the most commonly diagnosed solid cancer in males (1) and is currently the second leading cause of cancer-related deaths in men (2). Similarly to breast cancer, bone is the most common site for metastasis and its involvement is a major cause of morbidity. Approximately 90% of patients with advanced PCa have skeletal metastasis (3). Bone metastases can lead to pain, pathological fractures, nerve compression syndromes and hypercalcemia, with a significant decrease in quality of life (4). These complications result from excessive bone turnover, mainly bone formation which produces malignant bone lesions. While the principal characteristic of bone metastasis in PCa is the formation of osteoblastic lesions, bone absorption is initially needed to create the space for metastatic sites and to enhance bone formation. Therefore, therapies that effectively inhibit bone absorption are available for reducing the risk of skeletal complications in bone metastases of PCa, as well as those of other solid tumor types characterized by osteoclastic lesions (5).

*Bis*phosphonates (BPs) are potent inhibitors of osteoclast activity and survival, thereby reducing osteoclast-mediated bone absorption (6,7). Several studies have focused on the ability of BPs to inhibit tumorigenic cells, supporting the antitumoral and antimetastatic effects of BPs (8-10). Among the most potent nitrogen-containing BPs, zoledronic acid (ZA) has been demonstrated to exert a direct cytostatic and pro-apoptotic effect on PCa cell lines *in vitro* (11), to prevent cell adhesion and invasion *via* reduction of matrix metalloproteinase expression (12), and to inhibit testosterone-induced angiogenesis in a castrated animal model (13). Preclinical and clinical studies demonstrated anticancer benefits of ZA, although the molecular mechanism by which ZA prevents PCa cell growth remains unknown (14, 15).

Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, has been shown to inhibit apoptosis, enhance proliferation and promote angiogenesis (16).

Correspondence to: Martina Fragni, Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, V.le Europa 11, 25123 Brescia, Italy. Tel: +39 0303717515, Fax: +39 0303717529, e-mail: martina.fragni@unibs.it

Key Words: Prostate cancer, zoledronic acid, apoptosis, survivin, miR-203.

Although expressed at high levels during fetal development, survivin is rarely expressed in normal healthy adult tissues. It is, however, up-regulated in the majority of cancer types (17). Because of this up-regulation in malignancy, survivin is currently attracting considerable interest both as a potential cancer biomarker (18) and as new pharmacological target for cancer treatment (19).

MicroRNAs (miRNAs) are small regulatory non-coding RNAs that bind to specific target mRNAs, leading to translational repression of molecules involved in the regulation of biological processes, including cell growth, differentiation and apoptosis, both in physiological conditions and during diseases (20). The role of miRNAs in cancer development has been widely studied and has helped to elucidate events such as the change in expression of oncogenes, tumor suppressors and cancer-related proteins (21). In the specific field of tumorigenesis, miRNAs play an important role in mediating oncogenesis and favoring tumor progression as a result of their ability to modulate the epithelial-to-mesenchymal transition and other series of events facilitating the formation of metastases (22, 23). Previous studies demonstrated that *miR-203* is involved in the down-regulation of survivin protein expression (24) and it is considered an important regulator of cancer progression (25).

The aim of the present study was to analyze if survivin is a target of ZA, at both the transcriptional and the translational levels, in an attempt to elucidate the molecular mechanisms involved in the antiproliferative effect of ZA on PCa cells.

Materials and Methods

Cell lines. LNCaP, DU145 and PC3 cell lines were purchased from the American Type Culture Collection (ATCC) (LGC Promochem, Sesto San Giovanni, Milan, Italy) and maintained in culture, as suggested by the ATCC. Media and supplements were purchased from Euroclone (Pero, Italy). MycoFluor Mycoplasma Detection Kit (Life technologies Italia, Monza, Italy) was routinely used to test cells for mycoplasma contamination.

Drug. Injectable ZA (Zometa®) was obtained from Novartis Pharmaceuticals Corp (East Hanover, NJ, USA). Stock solutions were prepared from the hydrated disodium salt in saline solution. ZA-treated cells were exposed to different drug concentrations (1-100 μmol/l) for different times (up to 72 h) in serum-free medium (26, 27). Cells treated with saline solution were used as control.

Cell viability assay. Cells (5×10^4 cells/well) were treated with increasing concentrations of ZA (1-100 μmol/l) for 72 h. Cell viability was then evaluated by 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye reduction assay according to the manufacturer's protocol (Sigma Italia, Milano, MI, Italy). All experiments were performed at least three times run in triplicate.

Double staining with acridine orange (AO) and ethidium bromide (EtBr). Double staining with AO and EtBr was performed to visualize and quantify the number of viable, apoptotic and necrotic cells (28). Briefly, untreated and ZA-treated cells (5×10^5 cells/well) were centrifuged for 5 min at 700 × *g* and resuspended in phosphate-buffered saline. Dye mixture (100 μg/ml AO and 100 μg/ml EtBr) was then added to the cell suspension (1:10 final dilution) and cells immediately examined under a Zeiss LSM 510 META confocal laser-scanning microscope, with a ×10 objective (Carl Zeiss AG, Oberkochen, Germany). Several fields, randomly chosen, were digitized and about 1,000 nuclei for each sample were counted and scored using Image J software (NIH, Bethesda, MD, USA) (29).

Human apoptosis proteome profiler array. To examine the pathways through which ZA prompted apoptosis, we determined the apoptosis-related proteins utilizing the Proteome Profiler Human Array (R&D Systems, Space Import-Export, Milano, MI, Italy) in accordance with manufacturer's instructions. Briefly, untreated and ZA-treated LNCaP cells (4×10^6) were solubilized in the kit lysis buffer, with a complete set of protease and phosphatase inhibitors (Roche, Milan, Italy). Equal amounts of proteins (400 μg) from each sample were incubated with the human apoptosis array overnight at 4˚C. Finally, streptavidin–horseradish peroxidase and Chemi Reagent Mix were used to reveal apoptosis-related proteins by chemiluminescence. Pixel density was then analyzed by Image J software.

Western blot analysis. Untreated and ZA-treated cells (1×10^6 cells) were lysed in ice-cold buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.025% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% NP-40, 50 mM NaF, 2 mM phenylmethanesulfonyl fluoride), with protease and phosphatase inhibitor cocktails. Equal amounts of protein (30 μg) were separated by electrophoresis on a 4-12% NuPAGE Bis-Tris Gel System (Life Technologies) and electroblotted to a nitrocellulose membrane, following the manufacturer's instructions. A primary monoclonal antibody against human survivin was used at the final concentration of 2 μg/ml (Santa Cruz Biotechnologies, Heidelberg, Germany). A mouse monoclonal antibody direct against the *N*-terminal region of human α-tubulin (Sigma Italia) was applied to membranes, to normalize the values. A secondary anti-mouse antibody (final concentration 0.1 μg/ml; Santa Cruz Biotechnologies) was applied for 1 h at room temperature. The specific signal was visualized by the ECL-PLUS system (Amersham Italy, Milan, Italy). Densitometric analysis of the immunoblots was performed using a GelPro-Analyzer v 6.0 (MediaCybernetics, Bethesda, MD, USA).

RNA extraction, reverse transcription and quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from cells using the RNeasy kit (Qiagen, Milan, Italy) and 1 μg was transcribed into cDNA, using murine leukemia virus reverse transcriptase (Promega Italia, Milan, Italy). Gene expression was evaluated by qRT-PCR (iCycler iQ real-time PCR detection system; BioRad Laboratories, Milan, Italy), using SYBR Green as fluorochrome, as previously described (30). The sequences of sense and antisense oligonucleotide primers for survivin were: 5'-AGAACTGGCCCTTCTTGGAGG-3' and 5'-GTTTTTATGTTCCT CTATGGG-3', respectively (31), and for human β-actin were: 5'-TCTTCCAGCCTTCCTTCCCTG-3'; and 5'-CAATGCCAGGGTA CATGGTG-3', respectively. Reactions were performed under the following conditions: 1 cycle at 95˚C for 10 min, 40 cycles at 95˚C for 15 s, 62˚C for 1 min. Gene expression levels were normalized to those for β-actin expression. All reactions were performed in triplicate and data analyzed using the comparative Ct method (32).

Table I. *Prostate cancer cell death induction by zoledronic acid.*

	LNCaP	DU145	PC3
Viable cells	43.2±2%	38.9±0.5%	57.2±4%
Apoptotic cells	49.0±1%	58.6±2%	38.7±1%
Necrotic cells	7.8±0.4%	2.5±0.1%	4.1±0.1%

miRNA analysis. Total RNA, including miRNAs, was extracted from cells using the miRNeasy kit (Qiagen, Milan, Italy) and 1 μg was transcribed into cDNA using miScript II RT kit (Qiagen, Milan, Italy), following the manufacturer's protocol. qRT-PCR was performed with a miScript System (Qiagen, Milan, Italy) as elsewhere described (33). Gene expression was evaluated by iCycler iQ real-time PCR detection system (BioRad Laboratories), using SYBR Green as fluorochrome, as previously described (34). Reactions were performed under the following conditions: 15 min at 95˚C; 15s at 94˚C, 30 s at 55˚C, 30 s at 70˚C, for 40 cycles. The sequence of *miR-203* primer used was: 5'-GUGAAAUGUUUAG GACCACUAG-3'. Variations in expression of *miR-203* among different samples were calculated after normalization to U6.

Transfection of LNCaP cells with mimic miR-203. LNCaP cells were transfected with mimic *miR-203* using miScript mimic and HiPerFect as transfection reagent (Qiagen, Milan, Italy). The sequence of mimic *miR-203* was 5'-AGUGGUUCUUAACA GUUCAACAGUU-3'. Briefly, LNCaP cells (2×10^5 cells/well) were seeded in 6-well plate in growth medium without antibiotics for 24 h. Transfection complexes were prepared according to the manufacturer's instructions and delivered drop-wise onto the cells. After 72 h transfection, the expression level of survivin protein was measured by western blot assay.

Statistical analysis. Data analysis and graphics were obtained using GraphPad Prism 4 software (GraphPad Software, La Jolla, CA, USA). The statistical analysis was made using the one-way ANOVA, with a *post-hoc* test (Bonferroni's test) for multiple comparisons, considering $p<0.05$ as threshold for significant difference. Data are expressed as mean±SEM of at least three experiments run in triplicate, unless otherwise specified.

Results

ZA reduced proliferation of PCa cells. Exposure of cells to increasing concentrations of ZA caused a concentration-dependent reduction of cell proliferation (Figure 1). Time-course experiments demonstrated that the reduction of the viability reached its maximum at 72 h (data not shown). Sigmoidal concentration–response function was used to calculate the half-maximal inhibitory concentration (IC_{50}) value for ZA in LNCaP, DU145 and PC3 cell lines, that was: 19.4 μmol/l [95% confidence interval (CI)=18.48 to 20.31 μmol/l], 2.4 μmol/l (95% CI=2.15 to 2.61 μmol/l) and 41.44 μmol/l (95% CI=38.83 to 44.23 μmol/l), respectively. Based on these results, cells were treated with ZA at the IC_{50} value for 72 h in all subsequent experiments.

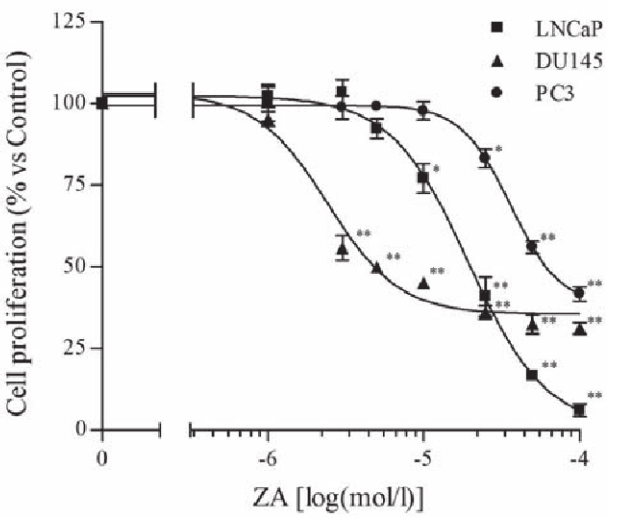

Figure 1. *Concentration-response curves of zoledronic acid (ZA) (1-100 μmol/l) in prostate cancer cells after incubation of 72 h. Results are expressed as the percentage of untreated cells. Data are the mean±SEM of three independent assays performed in triplicate. *p<0.01; **p<0.001 vs. control.*

ZA induced death of PCa cells. The ZA-mediated induction of cell death was observed using AO/EtBr double staining. Viable cells exhibited large green nuclei, whereas apoptotic cells (yellow) showed signs of nuclear condensation or nuclear bead formation. Necrotic cells displayed red nuclei without signs of nuclear condensation (Figure 2). Quantification of images demonstrated that ZA mainly induced cell death by activating apoptosis in PCa cells (Table I).

Survivin expression was down-regulated by ZA. In order to establish an overall perspective of the molecules involved in ZA cell death induction, we preliminary performed an array analysis of multiple apoptotic proteins in LNCaP cells after ZA exposure (Figure 3). Interestingly, among anti-apoptotic proteins analyzed, survivin was the most down-regulated and its expression was reduced by about 3.6-fold in ZA-treated cells compared to control. In order to study whether this effect on survivin expression was limited to LNCaP cells or a more general mechanism of action of ZA on PCa cells, survivin protein levels were analyzed by western blot in the three cell lines considered. We observed a significant decrease in survivin protein level of about 71.2±1% in LNCaP cells *vs.* untreated cells and expression became almost non-detectable in ZA-treated DU145 and PC3 cells (Figure 4). Regulation of survivin expression by ZA occurred at the transcription level, as ZA induced a significant reduction of survivin mRNA level (Table II).

ZA induced up-regulation of miR-203 expression. To assess survivin inhibition by ZA at the transcriptional level, *miR-*

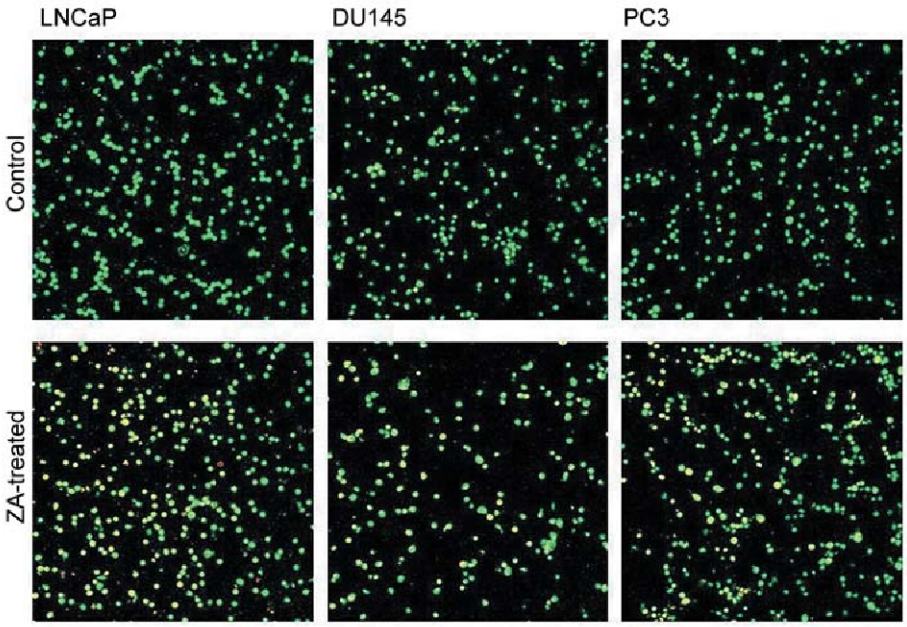

Figure 2. *Double staining of untreated and zoledronic acid (ZA) treated prostate cancer cells. Cells were stained using a mixture of acridine orange and ethidium bromide, as described in the Material and Methods section. Viable (green), apoptotic (yellow) and necrotic (red) cells were scored under a confocal laser-scanning microscope. Magnification, ×10.*

203 expression was analyzed in untreated and in ZA-treated cells. ZA significantly increased the relative level of *miR-203* in LNCaP cells (mean±SEM: 1.0±0.0 and 1.59±0.1 in untreated and treated cells, respectively) and in DU145 cells (mean±SEM: 1.0±0.0 to 1.4±0.03, respectively) (Figure 5a). PC3 cells were not analyzed in these experiments, as it is shown that *miR-203* expression is specifically attenuated in PCa cell lines derived from bone metastases (24). To strengthen the role of *miR-203* in regulating survivin mRNA expression, using LNCaP cells as an experimental model, a decrease of survivin protein of about 49.7±0.5% compared to non-transfected cells was observed in LNCaP cells transfected with mimic *miR-203* (Figure 5b).

Table II. *Down-regulation of survivin mRNA.*

	ΔCt	ΔΔCt	% *vs.* Untreated cells
LNCaP			
Untreated	7.4±0.2%	-	-
ZA-treated	8.3±0.1%	0.88	–46%*
DU145			
Untreated	6.7±0.1%	-	-
ZA-treated	7.3±0.1%	0.65	–36%*
PC3			
Untreated	5.6±0.2%	-	-
ZA-treated	6.4±0.2%	0.74	–41%*

*$p<0.01$.

Discussion

Although the main target of ZA is the osteoclast, there is increasing evidence for direct antitumor effects of ZA. Pre-clinical studies have shown that ZA inhibited tumor cell adhesion and invasion (35), induced tumor cell apoptosis (36), reduced cell viability (37) and exhibited anti-angiogenic effects (38). The mechanism of antitumor action of ZA, however, is still not completely understood (39), although the inhibition of the mevalonate pathway has been implicated (40).

In this study, we used different PCa cell lines to investigate the effects of ZA treatment on cell proliferation. Three worldwide recognized experimental cell models of PCa were used, namely: LNCaP cells, established from a lymph node localization, expressing an active mutated (T877A) androgen receptor (AR); DU145 cells, AR-negative and androgen-insensitive, derived from a brain metastasis; PC3, AR-negative and androgen-insensitive, derived from a bone metastasis.

Exposure of these cell lines to increasing concentrations of ZA induced a concentration-dependent decrease of cell viability, with a rank of potency of DU145 cells > LNCaP cells > PC3 cells. In particular, PC3 cells appeared to be the most resistant to ZA, as the IC_{50} was over 40 μmol/l, with still more than 50% of viable cells remaining. The different sensitivity of cells to ZA exposure is not surprising, as it has

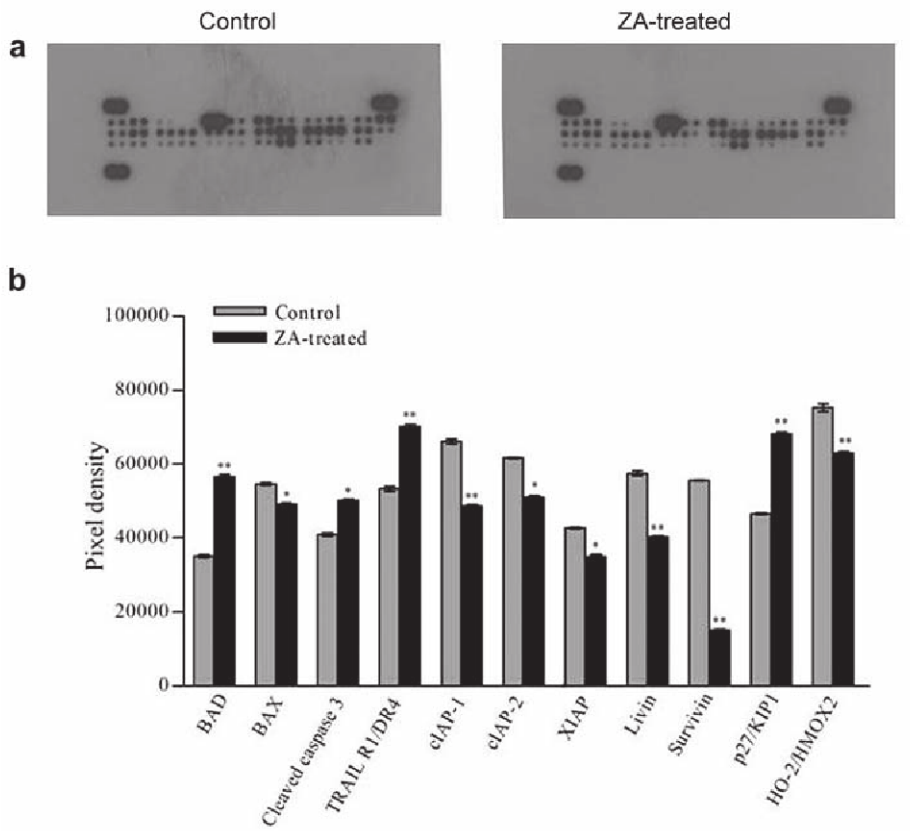

Figure 3. *a: Representative images of the apoptotic protein array are shown for the untreated and zoledronic acid (ZA)-treated LNCaP cells. b: Quantitative analysis of the arrays showed differences in the apoptotic markers between untreated and ZA-treated LNCaP cells. Only significant differences were considered. BAD: BCL2-associated death promoter, BAX: BCL2-associated X protein, TRAIL R1/DR4: tumor necrosis factor-related apoptosis-inducing ligand receptor 1/death receptor 4, cIAP-1: cellular inhibitor of apoptosis 1, cIAP-2: cellular inhibitor of apoptosis 2, XIAP: X-linked inhibitor of apoptosis, p27/KIP1: p27/kinase inhibitor protein 1, HO-2/HMOX2: heme oxygenase-2/inducible heme oxygenase-2. *p<0.01; **p<0.001 vs. control.*

already been demonstrated with other compounds (41) and it could be explained by the different phenotype and intracellular pathways activated in the different cell lines.

In accordance with others (42), we observed a concentration- and time-dependent reduction of proliferation in these cell lines, mainly due to apoptosis activation by ZA exposure. In the apoptosis array, we observed that both the intrinsic and the extrinsic apoptotic pathways seemed to be involved in cell death induction, as revealed respectively by an increase of BCL-2-associated death promoter (BAD) and tumor necrosis factor-related apoptosis-inducing ligand receptor 1/death receptor 4 (TRAIL R1/DR4) expression and a significant increase of p27/KIP1 protein involved in the cell-cycle arrest. Interestingly, the Human Apoptosis Proteome Profiler Array also showed a significant decrease in an anti-apoptotic protein group, the IAP family and our attention was drawn to survivin, which is one of the well-characterized members of this family (43). Currently, controversy exists over the precise mechanism by which IAP proteins inhibit apoptosis. However, evidence suggests that

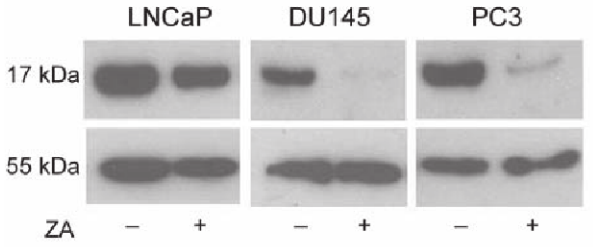

Figure 4. *Effects of zoledronic acid (ZA) treatment on survivin protein expression in prostate cancer cells, evaluated by western blot analysis. Human α-tubulin was used as internal control. The images are representative of three independent experiments.*

IAPs can directly inhibit caspases 3, 7 and 9 by proteasomal degradation (44). Survivin is the smallest IAP protein and it was shown to inhibit apoptosis both *in vitro* and *in vivo* (45), perhaps *via* interactions with multiple regulators of both intrinsic and extrinsic apoptosis pathways (46). Due to its role in many different cellular actions and signaling

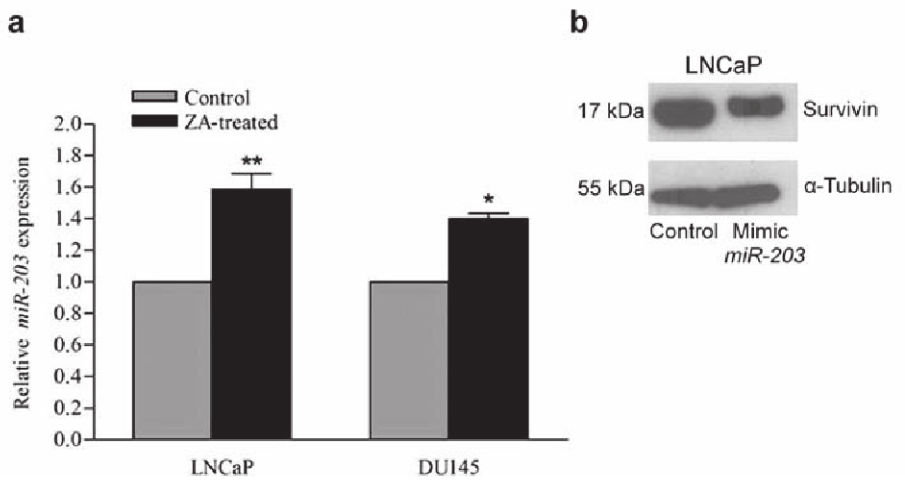

Figure 5. *a: Quantitative real-time polymerase chain reaction analysis of relative miR-203 expression levels in untreated and zoledronic acid (ZA)-treated cells. Data were normalized to U6 as an internal control and are represented as $2^{-\Delta\Delta Ct}$ mean±SEM. *p<0.01; **p<0.001 vs. control. b: Representative image of western blot assay for survivin in LNCaP cells transfected by mimic miR-203 and in non-transfected cells. Human α-tubulin was used as internal control.*

pathways, survivin has been described as a nodal protein. In addition to a role in suppressing apoptosis, survivin is also a mitotic regulator involved in various cell division processes (47). It has been believed for a long time that survivin is rarely expressed in healthy adult tissue, and only highly expressed during fetal development (48). Although recent reports suggest roles for survivin in normal cell (49) including T-cells (50), hematopoietic progenitor cells (51), vascular endothelial cells (52), gastrointestinal tract mucosa (53), erythroid cells (54) and polymorphonuclear cells (55), survivin expression is significantly higher in tumors, including PCa (56), thus suggesting a pathological role for the protein (57). Accordingly, we observed a major expression of *survivin* mRNA in PCa cells compared to PCS-440-010 human primary prostate epithelial cells (data not shown). Among IAP proteins modified by ZA exposure, survivin expression levels were the most significantly decreased. A previous study reported the decrease of survivin in ZA-mediated cytotoxicity in prostate cancer cells (58), but the molecular mechanism underlying the strong reduction of survivin expression after ZA treatment is still under investigation. Indeed, it can be due in part to inhibition of the mevalonate pathway, but it may also be caused by negative regulation at the gene expression level by ZA. Interestingly, an increasing amount of data suggests that inhibition of the mevalonate pathway may affect epigenetic regulation in cancer cells at the three most important levels: DNA methylation, histone deacetylation and miRNA (59). Over the last few years, most studies of the regulation of gene expression have focused mainly upon transcriptional activity; in particular, miRNA-mediated post-transcriptional regulation of protein-coding genes has emerged as a promising area of research (60). Some aberrantly expressed miRNAs have been discovered in PCa cell lines, xenografts and clinical tissues, and these PCa-related miRNAs may play a critical role in the pathogenesis of PCa (61). Among them, it was demonstrated that *miR-203* significantly reduced survivin in metastatic PCa cells (24). *miR-203* is an 'antimetastatic' miRNA that acts at multiple steps of the PCa metastatic cascade, including the epithelial–mesenchymal transition, cell invasion and motility, *via* repression of a cohort of pro-metastatic targets. Results reported here demonstrate that ZA reduced both the mRNA and protein expression of survivin in PCa cell lines, through the increase of *miR-203*.

In conclusion, the present study demonstrates that inhibition of survivin is associated with ZA-induced apoptosis of PCa cells, underlying the role of survivin as a new target for cancer therapeutics (62, 63), including PCa, as accumulating evidence indicates that survivin is associated with resistance to androgen deprivation in PCa (64).

Further studies are needed to determine future interventional implications of ZA as chemotherapeutic drug in addition to the well-consolidated treatment of bone metastases.

Conflicts of Interest

The Authors have no potential conflicts of interest in regard to this study.

Acknowledgements

This work was supported by the Fondazione Guido Berlucchi per la Ricerca sul Cancro Onlus (Borgonato, Bs, Italy) and by local grants from the University of Brescia.

References

1 Tao ZQ, Shi AM, Wang KX and Zhang WD: Epidemiology of prostate cancer: current status. Eur Rev Med Pharmacol Sci *19*: 805-812, 2015.

2 Bozzini G, Colin P, Nevoux P, Villers A, Mordon S and Betrouni N: Focal therapy of prostate cancer: energies and procedures.Urol Oncol *31*: 155-167, 2013.

3 Ye L, Kynaston HG and Jiang WG: Bone metastasis in prostate cancer: molecular and cellular mechanisms.Int J Mol Med *20*: 103-111, 2007.

4 Tanaka T, Kawashima H, Ohnishi K, Matsumura K, Yoshimura R, Matsuyama M, Kuratsukuri K and Nakatani T: Indirect antitumor effects of bisphosphonates on prostatic LNCaP cells co-cultured with bone cells.Anticancer Res *29*: 1089-1094, 2009.

5 Body JJ: Rationale for the use of *bis*phosphonates in osteoblastic and osteolytic bone lesions. Breast Suppl *2*: S37-S44, 2003.

6 Dumon JC, Journe F, Kheddoumi N, Lagneaux L and Body JJ: Cytostatic and apoptotic effects of bisphosphonates on prostate cancer cells. Eur Urol *45*: 521-528, 2004.

7 Oades GM, Coxon J and Colston KW. The potential role of bisphosphonates in prostate cancer.Prostate Cancer Prostatic Dis *5*: 264-272, 2002.

8 Freedman OC, Amir E and Clemons MJ: Adjuvant *bis*phosphonate therapy for breast cancer patients: Standard of care or future direction?Crit Rev Oncol Hematol *72*: 56-64, 2009.

9 Caraglia M, Marra M, Naviglio S, Botti G, Addeo R and Abbruzzese A: Zoledronic acid: an unending tale for an antiresorptive agent. Expert Opin Pharmacother *11*: 141-154, 2010.

10 Mystakidou K, Katsouda E, Parpa E, Kelekis A, Galanos A and Vlahos L: Randomized, open label, prospective study on the effect of zoledronic acid on the prevention of bone metastases in patients with recurrent solid tumors that did not present with bone metastases at baseline. Med Oncol *22*: 195-201, 2005.

11 Oades GM, Senaratne SG, Clarke IA, Kirby RS and Colston KW: Nitrogen-containing *bis*phosphonates induce apoptosis and inhibit the mevalonate pathway, impairing RAS membrane localization in prostate cancer cells. J Urol *170*: 246-252, 2003.

12 Coxon JP, Oades GM, Kirby RS and Colston KW: Zoledronic acid induces apoptosis and inhibits adhesion to mineralized matrix in prostate cancer cells *via* inhibition of protein prenylation. BJU Int *94*: 164-170, 2004.

13 Fournier P, Boissier S, Filleur S, Guglielmi J, Cabon F, Colombel M and Clézardin P: Bisphosphonates inhibit angiogenesis *in vitro* and testosterone-stimulated vascular regrowth in the ventral prostate in castrated rats. Cancer Res *62*: 6538-6544, 2002.

14 Neville-Webbe HL, Gnant M and Coleman RE: Potential anticancer properties of bisphosphonates. Semin Oncol Suppl *1*: S53-65, 2010.

15 Nogawa M, Yuasa T, Kimura S, Kuroda J, Segawa H, Sato K, Yokota A, Koizumi M and Maekawa T: Zoledronic acid mediates Ras-independent growth inhibition of prostate cancer cells. Oncol Res *15*: 1-9, 2005.

16 Ryan BM, O'Donovan N and Duffy MJ: Survivin: a new target for anticancer therapy.Cancer Treat Rev *35*: 553-562, 2009.

17 Li F: Survivin study: What is the next wave? Cell Physiol *197*: 8-29, 2003.

18 Duffy MJ, O'Donovan N, Brennan DJ, Gallagher WM and Ryan BM: Survivin: a promising tumor biomarker. Cancer Letter *249*: 49-60, 2007.

19 Altieri DC: Survivin, cancer networks and pathway-directed drug discovery. Nat Rev Cancer *8*: 61-70, 2008.

20 Lopez-Camarillo C, Marchat LA, Arechaga-Ocampo E, Perez-Plasencia C, Del Moral-Hernandez O, Castaneda-Ortiz EJ and Rodriguez-Cuevas S: MetastamiRs: non-coding microRNAs driving cancer invasion and metastasis. Int J Mol Sci *13*: 1347-1379, 2012.

21 Bouyssou JM, Manier S, Huynh D, Issa S, Roccaro AM and Ghobrial IM: Regulation of microRNAs in cancer metastasis. Biochim Biophys Acta *1845*: 255-265, 2014.

22 Ohtsuka M, Ling H, Doki Y, Mori M and Calin GA: MicroRNA processing and human cancer. J Clin Med *4*: 1651-1667, 2015.

23 Blandino G, Fazi F, Donzelli S, Kedmi M, Sas-Chen A, Muti P, Strano S and Yarden Y: Tumor suppressor microRNAs: A novel non-coding alliance against cancer. FEBS Lett *588*: 2639-2652, 2014.

24 Saini S, Majid S, Yamamura S, Tabatabai L, Suh SO, Shahryari V and Chen Y, Deng G, Tanaka Y and Dahiya R: Regulatory role of *mir-203* in Prostate cancer progression and metastasis.Clin Cancer Res *17*: 5287-5298, 2011.

25 Zhang M, Coen JJ, Suzuki Y, Siedow MR, Niemierko A, Khor LY, Pollack A, Zhang Y, Zietman AL, Shipley WU and Chakravarti A: Survivin is a potential mediator of prostate cancer metastasis.Int J Radiat Oncol Biol Phys *78*: 1095-1103, 2010.

26 Fromigue O, Lagneaux L and Body JJ: Bisphosphonates induce breast cancer cell death *in vitro*. J Bone Miner Res *15*: 2211-2221, 2000.

27 Skerjanec A, Berenson J, Hsu C, Major P, Miller WH Jr, Ravera C, Schran H, Seaman J and Waldmeier F: The pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with varying degrees of renal function. J Clin Pharmacol *43*: 154-162, 2003.

28 Atale N, Gupta S, Yadav UC and Rani V: Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. J Microsc *255*: 7-19, 2014.

29 Mironova EV, Evstratova AA and Antonov SM: A fluorescence vital assay for the recognition and quantification of excitotoxic cell death by necrosis and apoptosis using confocal microscopy on neurons in culture. J Neurosci Methods *163*: 1-8, 2007.

30 Sigala S, Bodei S, Missale C, Zani D, Simeone C, Cunico SC and Spano PF: Gene expression profile of prostate cancer cell lines: effect of nerve growth factor treatment. Mol Cell Endocrinol *284*: 11-20, 2008.

31 Liu C, Zhu Y, Lou W, Cui Y, Evans CP and Gao AC: Inhibition of constitutively active STAT3 reverses enzalutamide resistance in LNCaP derivative prostate cancer cells. Prostate *74*: 201-209, 2014.

32 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T) Method. Methods *25*: 402-408, 2001.

33 Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ and Zhu KM: Serum miR-146a and *miR-223* as potential new biomarkers for sepsis. Biochem Biophys Res Commun *394*: 184-188, 2010.

34 Johnson DJ, Andersen C, Scriven KA, Klein AN, Choi MR, Carroll C and de Leon RD: A molecular method to correlate bloodstains with wound site for crime scene reconstruction. J Forensic Sci *59*: 735-742, 2014.

35 Boissier S, Ferreras M, Peyruchaud O, Magnetto S, Ebetino FH, Colombel M, Delmas P, Delaissé JM and Clézardin P: *Bis*phosphonates inhibit breast and prostate carcinoma cell invasion, an early event in the formation of bone metastases. Cancer Res *60*: 2949-2954, 2000.

36 Senaratne SG, Pirianov G, Mansi JL, Arnett TR and Colston KW: Bisphosphonates induce apoptosis in human breast cancer cell lines. Br J Cancer *82*: 1459-1468, 2000.

37 Verdijk R, Franke HR, Wolbers F and Vermes I: Differential effects of *bis*phosphonates on breast cancer cell lines. Cancer Lett *246*: 308-312, 2007.

38 Wood J, Bonjean K, Ruetz S, Bellahcène A, Devy L, Foidart JM, Castronovo V and Green JR: Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. J Pharmacol Exp Ther *302*: 1055-1061, 2002.

39 Clézardin P, Benzaïd I and Croucher PI: Bisphosphonates in preclinical bone oncology. Bone *49*: 66-70, 2011.

40 Brown HK and Holen I. Antitumour effects of bisphosphonates in prostate cancer: What have we learned from *in vivo* models? Curr Cancer Drug Targets *9*: 807-823, 2009.

41 Sigala S, Faraoni I, Botticini D, Paez-Pereda M, Missale C, Bonmassar E and Spano P: Suppression of telomerase, re-expression of *KAI1*, and abrogation of tumorigenicity by nerve growth factor in prostate cancer cell lines. Clin Cancer Res *5*: 1211-1218, 1999.

42 Lee MV, Fong EM, Singer FR and Guenette RS: *Bis*phosphonate treatment inhibits the growth of prostate cancer cells. Cancer Res *61*: 2602-2608, 2001.

43 Salvesen GS and Duckett CS: IAP proteins: blocking the road to death's door. Nat Rev Mol Cell Biol *3*: 401-410, 2002.

44 Eckelman BP, Salvesen GS and Scott FL: Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO Rep *7*: 988-994, 2006.

45 Grossman D, Kim PJ, Blanc-Brude OP, Brash DE, Tognin S, Marchisio PC and Altieri DC: Transgenic expression of survivin in keratinocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. J Clin Invest *108*: 991-999, 2001.

46 Yamamoto T, Manome Y, Nakamura M and Tanigawa N: Down-regulation of survivin expression by induction of the effector cell protease receptor-1 reduces tumor growth potential and results in an increased sensitivity to anticancer agents in human colon cancer. Eur J Cancer *38*: 2316-2324, 2002.

47 Ambrosini G, Adida C, Sirugo G and Altieri DC: Induction of apoptosis and inhibition of cell proliferation by survivin gene targeting. J Biol Chem *273*: 11177-11182, 1998.

48 Adida C, Crotty PL, McGrath J, Berrebi D, Diebold J and Altieri DC: Developmentally regulated expression of the novel cancer anti-apoptosis gene survivin in human and mouse differentiation. Am J Pathol *152*: 43-49, 1998.

49 Fukuda S and Pelus LM: Survivin, a cancer target with an emerging role in normal adult tissues. Mol Cancer Ther *5*: 1087-1098, 2006.

50 Xing Z, Conway EM, Kang C and Winoto A: Essential role of survivin, an inhibitor of apoptosis protein, in T-cell development, maturation, and homeostasis. J Exp Med *199*: 69-80, 2004.

51 Fukuda S and Pelus LM: Regulation of the inhibitor-of-apoptosis family member survivin in normal cord blood and bone marrow CD34(+) cells by hematopoietic growth factors: implication of survivin expression in normal hematopoiesis. Blood *98*: 2091-2100, 2001.

52 Mesri M, Morales-Ruiz M, Ackermann EJ, Bennett CF, Pober JS, Sessa WC and Altieri DC: Suppression of vascular endothelial growth factor-mediated endothelial cell protection by survivin targeting. Am J Pathol *158*: 1757-1765, 2001.

53 Chiou SK, Moon WS, Jones MK and Tarnawski AS: Survivin expression in the stomach: implications for mucosal integrity and protection. Biochem Biophys Res Commun *305*: 374-379, 2003.

54 Gurbuxani S, Xu Y, Keerthivasan G, Wickrema A and Crispino JD: Differential requirements for survivin in hematopoietic cell development. Proc Natl Acad Sci USA *102*: 11480-11485, 2005.

55 Altznauer F, Martinelli S, Yousefi S, Thürig C, Schmid I, Conway EM, Schöni MH, Vogt P, Mueller C, Fey MF, Zangemeister-Wittke U and Simon HU: Inflammation-associated cell cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils. J Exp Med *199*: 1343-1354, 2004.

56 Rödel F, Sprenger T, Kaina B, Liersch T, Rödel C, Fulda S and Hehlgans S: Survivin as a prognostic/predictive marker and molecular target in cancer therapy. Curr Med Chem *19*: 3679-3688, 2012.

57 Altieri DC: Survivin, versatile modulation of cell division and apoptosis in cancer. Oncogene *22*: 8581-8589, 2003.

58 Sanli UA, Gorumlu G, Erten C, Gul MK, Cengiz E, Kucukzeybek Y, Karaca B, Atmaca H, Uzunoglu S, Karabulut B and Uslu R: Targeting apoptosis in the hormone- and drug-resistant prostate cancer cell line, DU-145, by gossypol/zoledronic acid combination. Cell Biol Int *33*: 1165-1172, 2009.

59 Karlic H, Thaler R, Gerner C, Grunt T, Proestling K, Haider F and Varga F: Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells. Cancer Genet *208*: 241-252, 2015.

60 Batkai S and Thum T: Analytical approaches in microRNA therapeutics. J Chromatogr B Analyt Technol Biomed Life Sci *964*: 146-152, 2014.

61 Shi XB, Tepper CG and White RW: MicroRNAs and prostate cancer.J Cell Mol Med *12*: 1456-1465, 2008.

62 Mita AC, Mita MM, Nawrocki ST and Giles FJ: Survivin: key regulator of mitosis and apoptosis and novel target for cancer therapeutics.Clin Cancer Res *14*: 5000-5005, 2008.

63 Mobahat M, Narendran A and Riabowol K: Survivin as a preferential target for cancer therapy. Int J Mol Sci *15*: 2494-2516, 2014.

64 Zhang M, Latham DE, Delaney MA and Chakravarti A: Survivin mediates resistance to antiandrogen therapy in prostate cancer. Oncogene *24*: 2474-2482, 2005.

Received December 15, 2015
Revised January 21, 2016
Accepted January 28, 2016