Bone Morphogenetic Protein-10 Suppresses the Growth and Aggressiveness of Prostate Cancer Cells Through a Smad Independent Pathway

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Purpose: BMPs have been implicated in the development of bone metastasis in prostate cancer. We investigated the role of BMP-10 in prostate cancer and prostate cancer cells.

Materials and Methods: BMP-10 expression was examined in human prostate tissue and prostate cancer cell lines. BMP-10 was experimentally over expressed in human prostate cancer cells. The influence of BMP-10 on the biological behavior of prostate cancer cells was then investigated in in vitro studies.

Results: BMP-10 expression was decreased or absent in prostate tumors, particularly in higher grade foci. Forced BMP-10 over expression in prostate cancer cells decreased in vitro growth, cell matrix adhesion, invasion and migration. Furthermore, BMP-10 induced apoptosis in prostate cancer cells through a Smad independent pathway, in which the 2 downstream candidates of BMP receptors XIAP (ILP) and ERK1/2 were activated. Interestingly the failure of BMP-10 to activate BMP receptor-II and the Smads in WT cells was due to the expression of BMP receptor-IB, which acted as a negative regulator of BMP receptor-II mediated Smad dependent signaling.

Conclusions: BMP-10 inhibits the growth of prostate cancer cells due largely to induced apoptosis via Smad independent signaling in which XIAP and ERK1/2 are involved. BMP-10 can also prevent prostate cancer cell migration and invasiveness. This suggests that BMP-10 may function as a tumor suppressor and apoptosis regulator for prostate cancer.

Key Words: prostate, prostatic neoplasms, bone morphogenetic proteins, apoptosis, neoplasm invasiveness

Bone is the most common metastatic site for advanced prostate cancer. These metastases often result in bone pain and pathological fracture, and cause spinal cord compression. BMPs, which are bone inductive factors enriched in the bone matrix, have been indicated in the development of prostate cancer, particularly in disease specific bone metastasis.1 We recently reported that BMP-9 functions as a putative tumor suppressor for prostate cancer.2 BMP-10 has 40.5% amino acid sequence identity with BMP-9 and it is mostly abundant in the trabeculae of the embryonic heart, whereas expression is weaker in the liver and the lungs. BMP-10-deficient mice die between days 9.5 and 10.5 of gestation due to a defect in cardiogenesis.3 Despite these observations of BMP-10 in development of the embryonic heart to our knowledge its role in prostate and cancer remains un-
known. In the current study the expression of BMP-10 was examined in prostate cancer. The biological function of BMP-10 in prostate cancer cells and potential signaling events were investigated.

MATERIALS AND METHODS

Materials

PC-3 (European Collection of Cell Cultures, Salisbury, United Kingdom), DU-145, LNCaP, CA-HPV-10, PZ-HPV-7 (ATCC®), PNT-1A and PNT-2C2 cells were used. Polyclonal anti-human BMP-10 was obtained from Orbigen, San Diego, California, while others were obtained from Santa Cruz Biotechnology, Santa Cruz, California. Secondary antibodies, reagents and kits were obtained from Sigma-Aldrich®. The table lists primer sequences.

Prostate tissue samples were snap frozen in liquid nitrogen immediately after radical prostatectomy, transurethral prostatectomy or prostate biopsy. All protocols were reviewed and approved by the local ethical committee and all patients gave written informed consent.

IHC Staining Procedure for Frozen Prostate Tissue

Eight prostate tumor and 13 normal tissues were sectioned using a cryostat and verified by 2 pathologists. IHC was performed using anti-BMP-10 antibody and a Vectastain® Universal Elite ABC kit.

Construction of BMP-10 Expressing and Ribozyme Transgenes, and Transfection

The full-length human BMP-10 coding sequence and hammerhead ribozymes targeting human BMP-10 was cloned into the mammalian expression plasmid vector pEF/His TOPO TA (Invitrogen™). The hammerhead ribozyme was designed and synthesized as described previously.4 BMP-10 expressing and ribozyme constructs with the verified insert and control plasmid vectors were then transfected into PC-3 cells, respectively, using electroporation. After 3 weeks of selection with blasticidin the transfected into PC-3 cells, respectively, using electroporation. After 3 weeks of selection with blasticidin the transfected into PC-3 cells, respectively, using electroporation.

Rh-BMP-10 Construction and Purification

The coding sequence of human BMP-10 with deletion of the stop codon was cloned into the same plasmid vector. rh-BMP-10 transgenes were transfected into murine fibroblast 3T3 cells. The recombinant protein was then purified using metal chelating affinity chromatography.

RNA Isolation and RT-PCR

RNA was isolated using Total RNA Isolation Reagent (ABgene®). RT-PCR was performed using standard methods.

Immunoprecipitation and Western Blot Analysis

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted. The blots were probed with respective specific antibodies and visualized using chemiluminescence detection. In case of immunoprecipitation after 2 hours of serum hunger the cells were exposed to rh-BMP-10 (20 ng/ml) or serum-free Dulbecco’s modified Eagle medium alone for 1 hour. The cells were lysed and immunoprecipitated using anti-phosphoserine/phosphothreonine antibody (Abcam®) and anti-phosphotyrosine antibody, respectively. The resulting immunoprecipitates and whole cell lysates were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel. After blotting proteins were probed using the corresponding antibody.

In Vitro Cell Growth Assay

A standard procedure was used, as we have previously described.5 Cells were plated into a 96-well plate at 2,500 cells per well. Cell growth was assessed after 1, 3 and 5 days. Crystal violet was used to stain cells and absorbance was determined at a wavelength of 540 nm using an Elx800™ spectrophotometer.

In Vitro Invasion Assay

The in vitro invasion assay was done as previously described.7 Transwell™ inserts with an 8 μm pore size were coated with 50 μg Matrigel™ and air dried. After rehydration 20,000 cells were added to each well. After 96 hours cells that had migrated through the matrix to the

<table>
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<th>PCR primer sequences</th>
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<td>1,275</td>
<td>58</td>
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other side of the insert were fixed, stained and counted under a microscope.

**Wounding Assay**
The wounding assay was performed as previously described. The monolayer of cells was scraped with a fine gauge needle. The movement of cells to close the wound was recorded on a time lapse video recorder and analyzed using OPTIMAS imaging analysis (Meyer Instruments, Houston, Texas).

**Cell Matrix Adhesion Assay**
The cell matrix adhesion assay was done as previously described. Cells were added to each well of 96-well plate precoated with Matrigel (5 μg per well). After 40 minutes of incubation nonadherent cells were washed off using balanced salt solution buffer. The remaining adherent cells were fixed, stained and counted.

**Flow Cytometric Analysis of Apoptosis**
All cells, including those floating in culture medium, were harvested after an incubation period. The apoptotic population of the cells was determined using a Vybrant® Apoptosis Assay Kit and flow cytometry with a CyFlow®.

**p-Smad-1 and Caspase-3 Immunofluorescence Staining**
Following 2 hours of incubation in serum-free Dulbecco’s modified Eagle medium cells were exposed to rh-BMP-10 (40 ng/ml) or serum-free medium alone for 1 hour. Immunofluorescence staining of p-Smad-1 was performed using anti-phospho-Smad1 antibody and tetramethylrhodamine isothiocyanate labeled anti-goat IgG.

PC-3 cells were treated with rh-BMP-10 (40 ng/ml) for up to 24 hours. The cells were then fixed and probed using anti-active caspase-3 (Abcam) and FITC labeled secondary antibody. Nuclei were counterstained using Hoechst stain.

**Statistical Analysis**
Statistical analysis was performed using Minitab®, version 14. The Mann-Whitney test was used for nonnormally distributed data and the 2-sample t test was used for normally distributed data. Analysis of positive IHC stain-
ing in prostate tissues was performed using Fisher's exact test with differences considered statistically significant at \( p < 0.05 \).

**RESULTS**

**BMP-10 Expression in Prostate Tissues and Cell Lines**

PC-3 was initially derived from bone metastasis, DU-145 was derived from brain metastasis and LNCaP was derived from lymph node metastasis, whereas CA-HPV-10 was derived from a primary tumor. PZ-HPV-7, PNT-1A and PNT2-C2 cells are immortalized prostatic epithelial cells. The BMP-10 transcript was detectable only in DU-145 but not in the 3 prostatic epithelial cell lines (fig. 1, A). This may have been due to the change in phenotype in vitro. ALK-1 was not detectable in any of these cell lines. Other type I BMP receptors and type II receptor transcripts were detected in all cell lines (fig. 1, A).

BMP-10 mRNA expression was detected in 2 of the 3 normal prostate tissues but it was weakly expressed in 1 of the prostate cancer tissues examined (fig. 1, B). BMP-10 protein expression was also examined in human prostate tissues. BMP-10 staining was seen in normal prostatic epithelium but it decreased or was absent in prostate cancer cells, particularly at high grade loci, where the gland structure was completely disrupted (fig. 2). Positive staining was significantly different in normal vs tumor tissue (11 of 13 or 84.6% vs 1 of 8 or 12.5%, Fisher's exact test \( p = 0.0022 \)).

**Influence of BMP-10 Over Expression on Prostate Cancer Cellular Biology**

BMP-10 over expression was successfully established in PC-3\(^{BMP-10exp}\) cells after transfection compared with that in WT (PC-3\(^{WT}\)) and control transfection PC-3\(^{pEF/His}\) cells (fig. 3).

We first determined the effect of BMP-10 over expression on in vitro cell growth. An inhibitory

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**Figure 2.** BMP-10 in prostate specimens and cell line. A and E, normal prostate. B and F, Gleason score 7 prostate cancer. C and G, Gleason score 9 prostate cancer. D, DU-145 cells as positive control. H, negative control using secondary antibody. A to D, IHC staining, reduced from \( \times 100 \). E to H, H & E, reduced from \( \times 100 \).

**Figure 3.** Forced over expression of BMP-10 in prostate cancer cells. A, RT-PCR reveals BMP-10 transcript. B, Western blot shows BMP-10 protein.
effect on cell growth by BMP-10 over expression was seen in prostate cancer cells (fig. 4). This was consistent with observations in DU-145\textsuperscript{ΔBMP-10} cells, in which BMP-10 expression had been knocked down (fig. 5, A). Cell growth was marginally increased in the DU-145\textsuperscript{ΔBMP-10} cell line after the loss of endogenous BMP-10 compared to that in control cells (fig. 5, B).

We further examined the influence of BMP-10 on prostate cancer cell invasive and motile features.

Figure 4. In vitro assay results of influence of BMP-10 over expression on prostate cancer cell biological behavior. A, BMP-10 inhibited PC-3 cell growth. Decreased cell growth was seen at day 5 in PC-3\textsuperscript{BMP-10exp} vs PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His} cells. B, BMP-10 influence on invasiveness. C, BMP-10 decreased prostate cancer cell adhesion. Error bars indicate SD. Asterisks indicate p <0.01 vs PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His}.

Figure 5. BMP-10 knockdown influence on DU-145 cell growth. A, RT-PCR shows BMP-10 knockdown using hammerhead ribozyme transgenes. B, in vitro assay demonstrates BMP-10 knockdown effect on cell growth. ∆ indicates p <0.05 vs DU-145\textsuperscript{pEF} control.

Figure 6. In vitro wounding assay reveals influence of BMP-10 over expression on prostate cancer cell biological behavior. Over expression inhibited PC-3 cell migration. Asterisk indicates p <0.05 vs PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His}.
Figure 7. BMP-10 induced PC-3 cell apoptosis. A, Western blot shows that rh-BMP-10 was purified from 3T3 BMP-10 transfectants. B, rh-BMP-10 inhibited PC-3 cell growth.

Figure 8. BMP-10 induced apoptosis in PC-3 cells. Flow cytometry shows increased apoptotic PC-3 cell population 24 hours after exposure to 40 ng/ml rh-BMP-10. Incidence of cells undergoing apoptosis after rh-BMP-10 exposure was 23.90% vs 7.73% in control.
PC-3\textsuperscript{BMP-10\exp} cells had markedly increased invasiveness compared to that of PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His} cells (fig 4, B). Similarly BMP-10 over expression inhibited the cell matrix adhesion of prostate cancer cells compared with that of PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His} cells (fig 4, C). PC3\textsuperscript{BMP10\exp} cells also showed increased cellular migration 90 minutes after wounding compared with that of controls (fig. 6).

**BMP-10 Induced Apoptosis in Prostate Cancer Cells In Vitro**

rh-BMP-10 was purified from transfected 3T3 cells (fig. 7, A). Biological activity of rh-BMP-10 was examined using the in vitro growth assay (fig. 7, B). Cell growth was inhibited after 3 days of exposure to rh-BMP-10. Inhibition was seen in a concentration dependent manner.

We further determined the possible apoptotic effect of BMP-10 in PC-3 cells. We chose 40 ng/ml for the current study based on the growth assay and previous reports.\textsuperscript{9–12} A remarkable increase in the apoptotic population in PC-3 cells was induced by rh-BMP-10 after 24 hours of exposure (fig. 8). Caspase-3, an effector of extrinsic apoptosis cascade, was also examined in PC-3 cells upon exposure to rh-BMP-10. Caspase-3 activation was seen in PC-3 cells after 4 hours of exposure to rh-BMP-10 (fig. 9).

**Role of BMPR-IB and II, and R-Smads in PC-3 Cell BMP-10 Signaling**

To investigate the involvement of BMP receptors in the signal transduction of BMP-10 in prostate cancer cells we used PC-3\textsuperscript{ABMPR-IB} and PC-3\textsuperscript{ABMPR-II} cells in which BMPR-IB and BMPR-II, respectively, had been knocked down (fig. 10, A and B).\textsuperscript{2} Phosphorylation of these 2 receptors by BMP-10 was then examined in these cells. There was no BMPR-IB phosphorylation in the cells following treatment with rh-BMP-10. BMPR-II was phosphorylated by BMP-10 in the absence of BMPR-IB in PC-3\textsuperscript{ABMPR-IB} cells compared to that in PC-3\textsuperscript{WT} and PC-3\textsuperscript{pEF/His} cells, in which no phosphorylation was seen (fig. 10, C). This suggests that the existence of BMPR-IB negated BMPR-II activation in PC-3 cells.

To elucidate Smad dependent signaling by BMP-10 in PC-3 cells we examined the activation of R-Smads 1, 5 and 8. There was no noticeable activation of Smads 1, 5 and 8 in cells treated with rh-BMP10 (fig. 10, D), including PC-3\textsuperscript{WT}, PC-3\textsuperscript{pEF/His} and PC-3\textsuperscript{ABMPR-II} cells. Most interestingly there was a significantly increased level of phosphorylated Smad-1 induced by BMP-10 in PC-3\textsuperscript{ABMPR-IB} BMPR-IB knockdown cells (fig. 10, D). Smad-1 activation and the consequent translocation to nuclei of phosphorylated Smad-1 was seen only in PC-3\textsuperscript{ABMPR-IB} cells as the result of exposure to rh-BMP-10 (fig. 10, E). However, the same activation

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**Figure 9.** BMP-10 induced PC-3 cell apoptosis. A, control. B, active caspase-3 staining (arrows) using FITC was increased in PC-3 cells exposed to 40 ng/ml rh-BMP-10 for 4 hours compared to that in control. C, nuclei of cells treated with BMP-10 were counterstained with Hoechst 33258. D, merged image of FITC labeled (arrows) active caspase-3 and Hoechst stained preparations.
Potential Signaling Events Downstream of BMP-10 Induced Smad Independent Pathway in PC-3 Cells

PC-3 cells were exposed to rh-BMP-10 for up to 2 hours and serine/threonine or tyrosine phosphorylated proteins were then examined using immunoprecipitation and Western blot analysis (fig. 11). Serine/threonine phosphorylation of ILP (XIAP), an adaptor for BMP receptor signaling through the Smad independent pathway, was seen after 5 minutes of exposure to rh-BMP-10. The downstream mediating molecule TAK1 was also activated via serine/threonine phosphorylation, which is involved in the trigger of MAPK cascades. Three members of the MAPK family, including p38, ERK1/2 and JNK, were examined in the current experiment. An increase in ERK1/2 serine/threonine phosphorylation was seen in PC-3 cells after 1 hour of exposure to rh-BMP-10. Marginal phosphorylation of p38 on tyrosine residues was also seen. No obvious activation of JNK and p38 (serine/threonine phosphorylation) was noted (fig. 11).

DISCUSSION

In the current study we first noted that BMP-10 expression was decreased in prostate cancer and we identified a potential anticancer role of BMP-10 in prostate cancer cells. BMP-10 over expression in prostate cancer cells had broad inhibitory effects on cell growth, adhesion and motility. A key change in cellular function was the decrease in growth. We further observed that the inhibitory effect of BMP-10 on cell growth was achieved through the induction of caspase-3 involved apoptosis.

It was recently reported that ALK1, BMPR-IA (ALK3) and BMPR-IB (ALK6) are candidate type I receptors for BMP-10 and BMPR-II, and activin type
IIA receptor is the candidate type II receptor. ALK-1 transcript is not detectable in prostate cancer or prostatic epithelial cell lines, particularly in PC-3 cells, which were used in the current study. Of these receptors BMPR-IB and BMPR-II have been implicated in prostate cancer progression. However, to our knowledge whether these 2 receptors are concurrently involved in BMP-10 signal transduction in prostate cancer is unknown. We have reported the transcripts of these 2 receptors expressed in all 7 prostate cell lines. The involvement of these 2 receptors, and R-Smads 1, 5 and 8 in signaling by BMP-10 in PC-3 cells was first examined using PC-3 cells in which BMPR-IB and BMPR-II, respectively, had been knocked down. There was no obvious activation of either receptor in PC-3 cells after exposure to BMP-10. However, phosphorylation of BMPR-II serine/threonine residues was seen in PC-3BMPR-IB cells after the loss of BMPR-IB. This led to the subsequent activation and nuclear translocation of Smad-1, and resulted in the transcriptional regulation of target genes. This is in contrast to signaling by BMP-9, a close member of BMP-10 in the BMP family, which induces apoptosis in prostate cancer cells through a Smad dependent pathway. This demonstrates that BMPR-IB in PC-3 cells can prevent BMPR-II phosphorylation, thus influencing the consequent activation of Smad-1. It suggests that BMP-10 signaling in PC-3 cells is more likely achieved through a Smad independent pathway. The presence of BMPR-IB in PC-3 cells can inhibit the phosphorylation of Smad-1 induced by BMP-10, preventing its signaling through the Smad dependent pathway. To our knowledge whether receptors other than ALK-1, BMPR-IB and BMPR-II are involved in BMP-10 Smad independent signal transduction remains to be elucidated.

Some intracellular signaling molecules involved in Smad independent signal transduction by BMPs were further investigated. Phosphorylation of ILP (XIAP) serine/threonine residues was identified in PC-3 cells after 5 minutes of exposure to rh-BMP-10. ILP acts as an adaptor protein that links the receptors to TAK1 binding protein 1 and TAK1 in the BMP signaling pathway. TAK1 activation has been implicated in the apoptosis induced by BMP-2 and transforming growth factor-β1. TAK1 inactivation could block BMP-2 induced apoptosis.

We also examined the involvement of MAPKs, which have been implicated in the apoptotic cascades downstream of TAK1 activation, particularly p38 and JNK. However, neither p38 nor JNK was activated in PC-3 cells when exposed to BMP-10. ERK1/2 phosphorylation was noted in PC-3 cells after 1 hour of exposure to rh-BMP-10. ERK1/2 activation has been demonstrated to lead to anti-apoptotic and pro-apoptotic actions. For example, BMP-2 can protect breast cancer cells from the apoptosis induced by hypoxia by ERK1/2 activation. On the other hand, ERK1/2 has also been implicated in the activation or promotion of apoptosis, for example ERK1/2 tyrosine phosphorylation has a key role in mediating the apoptosis induced by oxidative stress. ERK1/2 can elicit apoptosis in epithelial cells by activating caspase-3 during oxidant injury. This is consistent with observations in the
current study. We propose the signaling pathway downstream of BMP-10 (fig. 12).

CONCLUSIONS
Taken together BMP-10 suppresses the growth, adhesion, invasion and migration of prostate cancer cells, at least in part through the Smad independent XIAP-TAK1-ERK pathway. This suggests that it may function as tumor suppressor for prostate cancer.

ACKNOWLEDGMENTS
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REFERENCES
8. Jiang WG, Hiscox SE, Parr C, Martin TA, Matsu-
moto K, Nakamura T et al: Antagonistic effect of
NK4, a novel hepatocyte growth factor variant, on in vitro angiogenesis of human vascular endo-

9. Abdelaal MM, Tholpady SS, Kessler JD, Morgan

10. Brown MA, Zhao Q, Baker KA, Naik C, Chen C,
Pukac L et al: Crystal structure of BMP-9 and
functional interactions with pro-region and recep-

Bezooijen RL, Zhao Q, Pukac L et al: BMP-9
signals via ALK1 and inhibits bFGF-induced endo-
thelial cell proliferation and VEGF-stimulated an-

12. Buggisch M, Ateghang B, Ruhe C, Strobel C,
Lange S, Wartenberg M et al: Stimulation of
ES-cell-derived cardiomyogenesis and neonatal cardiac cell proliferation by reactive oxygen spe-

13. Mazenbourg S, Sangkuh K, Luo CW, Sudo S,
Klein C, Hsuieh AJ: Identification of receptors and
signaling pathways for orphan bone morphoge-
netic protein/growth differentiation factor ligands
280: 32122.

14. David L, Mallet C, Mazenbourg S, Feige JJ and
Bailly S: Identification of BMP9 and BMP10 as
functional activators of the orphan activin recep-
tor-like kinase 1 (ALK1) in endothelial cells. Blood

15. Kim IV, Lee DH, Ahn HJ, Tokunaga H, Song W,
Deveraux LM et al: Expression of bone morpho-
genetic protein receptors type-IA, -IB and -II cor-
relates with tumor grade in human prostate can-

SJ et al: Loss of expression of bone morphoge-
netic protein receptor type II in human prostate

17. Ide H, Katoh M, Sasaki H, Yoshida T, Aoki K,
Nawa Y et al: Cloning of human bone morpho-
genetic protein type IB receptor (BMPR-IB) and its
expression in prostate cancer in comparison with
other BMPRs. Oncogene 1997; 14: 1377.

18. Yamaguchi K, Nagai S, Ninomiya-Tsuji J, Nishita
M, Tamai K, Irie K et al: XIAP, a cellular member of
the inhibitor of apoptosis protein family, links the
receptors to TAB1-TAK1 in the BMP signaling

19. Yamaguchi K, Shirakabe K, Shibuya H, Irie K,
Oishi I, Ueno N et al: Identification of a member
of the MAPKKK family as a potential mediator of
270: 1645.

and Taka T: BMP2-induced apoptosis is mediated
by activation of the TAK1-p38 kinase pathway
that is negatively regulated by Smad6. J Biol

21. Edlund S, Bu S, Schuster N, Aspenstrom P,
Heuchel R, Heldin NE et al: Transforming growth
factor-beta1 (TGF-beta)-induced apoptosis of
prostate cancer cells involves Smad7-dependent
activation of p38 by TGF-beta-activated kinase 1
and mitogen-activated protein kinase kinase 3.
Mol Biol Cell 2003; 14: 529.

22. Raida M, Clement JH, Ameri K, Han C, Leek RD
and Harris AL: Expression of bone morphogenetic
protein 2 in breast cancer cells inhibits hypoxic

23. Lee YJ, Cho CN, Soh JW, Jhon GJ, Cho CK,
Chung HY et al: Oxidative stress-induced apopto-
sis is mediated by ERK1/2 phosphorylation. Exp

24. Zhuang S, Yan Y, Daubert RA, Han J and Schnell-
mann RG. ERK promotes hydrogen peroxide-in-
duced apoptosis through caspase-3 activation
and inhibition of Akt in renal epithelial cells.