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Interaction of testisin with maspin and its impact on invasion and cell death resistance of cervical cancer cells

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ABSTRACT

Previous studies have shown that testisin promotes malignant transformation in cancer cells. To define the mechanism of testisin-induced carcinogenesis, we performed yeast two-hybrid analysis and identified maspin, a tumor suppressor protein, as a testisin-interacting molecule. The direct interaction and cytoplasmic co-localization of testisin with maspin was confirmed by immunoprecipitation and confocal analysis, respectively. In cervical cancer cells, maspin modulated cell death and invasion; however, these effects were inhibited by testisin in parallel experiments. Of interest, the doxorubicin resistance was dramatically reduced by testisin knockdown ($P = 0.016$). Moreover, testisin was found to be over-expressed in cervical cancer samples as compared to matched normal cervical tissues. Thus, we postulate that testisin may promote carcinogenesis by inhibiting tumor suppressor activity of maspin.

Structured summary:
MINT-7712215, MINT-7712176: *Testisin* (uniprotkb:Q9Y6M0) *binds* (MI:0407) to *Maspin* (uniprotkb:P36952) by *pull down* (MI:0096)
MINT-7712188: *Testisin* (uniprotkb:Q9Y6M0) and *Maspin* (uniprotkb:P36952) *colocalize* (MI:0403) by *fluorescence microscopy* (MI:0416)
MINT-7712115: *Testisin* (uniprotkb:Q9Y6M0) *physically interacts* (MI:0915) with *Maspin* (uniprotkb:P36952) by *two-hybrid* (MI:0018)
MINT-7712162, MINT-7712128: *Maspin* (uniprotkb:P36952) *physically interacts* (MI:0915) with *Testisin* (uniprotkb:Q9Y6M0) by *anti bait co-immunoprecipitation* (MI:0006)
MINT-7712147: *Testisin* (uniprotkb:Q9Y6M0) *physically interacts* (MI:0915) with *Maspin* (uniprotkb:P36952) by *anti tag co-immunoprecipitation* (MI:0007)

1. Introduction

Testisin (also known as PRSS21 and ESP-1) is a serine protease that is highly expressed in various tumor cell lines but shows little expression in normal tissues, with the exception of testicular germ cells [1–3]. Previous studies have shown that overexpression of testisin induces colony formation and promotes malignant transformation in ovarian cancer cells [4]. However, the action mechanism or physiologic substrates for testisin have not been previously determined. Using a yeast-based screening system and a HeLa-derived cDNA library, we identified maspin as a novel testisin-interacting molecule.

Maspin is a type II tumor suppressor that has sequence homology with members of serpin family of protease inhibitors [5]. In many different cancers, maspin acts as a tumor suppressor capable of inhibiting motility, invasion and metastasis [6–8]. Consistent with this tumor suppressor function, impaired expression of maspin has been reported in several epithelial-type human malignancies, including breast, prostate and lung cancer [6,9]. In a mammary tumor model, a strong correlation was found between maspin overexpression and increased cell death, suggesting that maspin may play an important role in cell death in vivo [10]. Maspin was found to sensitize cancer cells to chemical induction of

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apoptosis [11,12], and in a transgenic mouse model, maspin was found to inhibit tumor progression in vivo through a combination of increased apoptosis, decreased angiogenesis, and inhibition of tumor cell migration [10]. However, significant in vitro and in vivo tumor growth variations have been noted among cells that stably express maspin [13].

Here, we show that maspin is a novel target of testisin. Although testisin proteins have previously been associated with transformation, this is the first study to show how testisin modulate the apoptosis and invasion of cancer cells.

2. Materials and methods

2.1. Cell culture and transfection

Human cervical carcinoma CaSki cells were grown in RPMI (Gibco Life Science, Grand Island, NY). SiHa, MS-751, and HeLa cells were grown in DMEM (Gibco Life Science). All media were supplemented with 10% FBS, 1 mM $NaCO_3$, 2 mM L-glutamine, penicillin–streptomycin, and cells were grown in 5% CO_2 at 37 °C. Maspin- and testisin-targeting siRNA and control siRNA were obtained from Dharmacon (Lafayette, CO). All transfections were performed using Effectine (Qiagen, Valencia, CA) according to the manufacturer's instructions.

2.2. Yeast two-hybrid analysis

The testisin bait sequence was amplified from full-length wild type human testisin cDNA and inserted into pLexA DNA-binding domain (pBD; Clontech, Palo Alto, CA) to make the bait construct. The reporter strain EGY48 (Clontech) was sequentially transfected with pBD-testisin and the pB42 AD-tagged HeLa cDNA library. Screening was performed according to the protocol provided with the Matchmaker Two-Hybrid System (Clontech), and positive clones were selected on supplemented minimal galactose medium. To double-check the positive colonies, qualitative blue-white screening with 40 μg/ml 5-bromo-4-chloro-3-indolyl-D-galactopyranoside (X-gal) was performed. Controls consisted of pJG4-5/Daxx and pLexA/sentrin in EGY48 (positive control; P) and pJG4-5/FADD and LexA/sentrin (negative control; N) were streaked on S.D. medium lacking uracil and histidine [14].

2.3. Constructs of expression plasmids for testisin and maspin

All constructs were generated by PCR using primers designed from the coding regions of the relevant human cDNAs. The full-length testisin open reading frame was cloned from HeLa mRNA by reverse transcription-PCR (RT-PCR) for FLAG-tagged cloning into pCMVTaq4C (Clonetech). Myc-tagged-maspin constructs were ligated into pCDNA3.1 (Clonetech).

2.4. RT-PCR analysis of maspin and testisin

Expression of maspin and testisin was evaluated by RT-PCR. The primer sequences, which were designed from the coding region of the human maspin cDNA, were as followings: 5′-AGGCCTTACATGGTGTGACTCCAT-3′ (sense) and 5′-GATTTATGCCCCACTCTGTCCCTA-3′ (antisense). The PCR conditions were as follows: 27 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s, followed by a final incubation at 72 °C for 7 min. The testisin primer sequences, which were designed from the coding region of the human testisin cDNA, were as follows: 5′-CTTAAGCTTATGGGCGCGCGCGGG-3′ (sense) and 5′-CAACTCGAGTTAGACCGGCCCCAGGAG-3′ (antisense). The PCR conditions were as follows: 27 cycles of 95 °C for 30 s, 58 °C for 60 s, and 72 °C for 40 s, followed by a final incubation at 72 °C for 7 min.

2.5. In vitro transcription, translation, and GST pull-down assay

The maspin cDNA was in-frame cloned into pGEX4T-1 (Amersham Corp., Arlington Heights, IL). Glutathione S-transferase (GST) fusion proteins were expressed in *Escherichia coli* BL21 (DE3) with IPTG (isopropyl-B-D-thiogalactopyranoside) induction. Subsequently cells were sonicated in ice-cold lysis buffer (200 mM Tris–Cl, pH 8.0, 0.5 M NaCl, 100 mM EDTA, 0.1% Triton X-100, 0.4 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Recombinant GST fusion proteins were recovered by incubation with glutathione-Sepharose beads (Amersham Pharmacia Biotech) for 16 h at 4 °C and normalized for protein concentration. The testisin cDNA was subcloned into pcDNA3.1(+) (Invitrogen, Carlsbad, CA) and the resulting pcDNA3.1(+)/testisin were subjected to in vitro translation using the TNT transcription/translation system kit (Promega, Madison, WI). GST pull-down assay was performed as previously described [14].

2.6. Co-immunoprecipitation and immunoblotting

To test for an association between endogenous maspin and testisin, cells (or tissue) were washed with phosphate-buffered saline (PBS) and lysed in buffer (20 mM HEPES, pH 7.0, 150 mM NaCl, 1 mM EDTA, 2 mM β-glycerophosphate, 1% Triton X-100, 10% glycerol, 1 mM PMSF, 1X protease inhibitor cocktail). The cell (or tissue) debris was removed by centrifugation at 13 000 rpm for 15 min at 4 °C, and the resulting supernatant was incubated with anti-maspin (1:100, Santa Cruz, CA) or anti-FLAG (Sigma, St. Louis, MO) antibody for 16 h at 4 °C on a rotary shaker. Subsequently, immune complexes were washed with the above-mentioned lysis buffer, boiled for 3 min in 2X SDS loading buffer, and resolved by 10% SDS–PAGE. The proteins were then transferred to a nitrocellulose blot overnight at 4 °C, and developed against anti-FLAG, testisin (Abnova, Taipei, Taiwan), and maspin antibodies.

2.7. Confocal microscopy

Cells were grown on four-well Lab-Tek Chamber Slide Glass (Nunc, Scotts Valley, CA) and fixed with 3.7% formaldehyde for 15 min at room temperature. Fixed cells were then incubated with primary antibodies overnight, washed with PBS, and incubated with the secondary antibodies conjugated to either rhodamine or FITC. DAPI was used to counterstain the nuclei. Confocal scanning analysis of the cells was done with RADIANCE 2100 confocal imaging system (Bio-Rad, Hercules, CA).

2.8. Caspase-3 activity assay and cell death evaluation

Cells (1×10^5) were plated to six-well plate and evaluated for caspase-3 activity using a caspase-3 activity assay kit from Peptron (Seoul, Korea) according to the manufacturer's instructions. For cell death evaluation, cells were stained with trypan blue solution, and the blue-stained dead cells were counted under microscope. The percent of dead cell was calculated as the number of blue-stained cells/number of total cells counted. Values represent the averages of three independent experiments.

2.9. Cell viability analysis

Cells (5000 cells/well) were incubated in triplicate on a 96-well plate in the presence or absence of the indicated test samples in a final volume of 0.1 ml for 24 h at 37 °C. Thereafter, 0.025 ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (5 mg/ml in PBS) was added to each well. MTT assay was performed as previously described [15].

2.10. Invasion assay

Cancer cells were plated at 5×10^4 cells/well in RPMI medium with 1% serum in the upper chamber of a Transwell insert (8-μm pores; Chemicon, Temecula, CA) coated with Matrigel. Medium containing 10% serum was added to the bottom chamber, and the chamber was incubated for 24 h. The cells in the upper chamber were then removed by scraping, the cells remaining on the lower surface of each insert were stained using CyQuant GR dye (Chemicon), and cell numbers were counted.

2.11. Statistical analysis

Unless otherwise stated, the data are reported as the mean ± S.D. from a representative experiment. The *t*-test was used for comparing results, and statistical significance was based on $P < 0.05$. All experiments were performed at least three times, each time with three or more independent observations.

3. Results

3.1. Testisin interacts with maspin

In order to identify an intracellular target of testisin, we searched for testisin binding proteins in a human HeLa cDNA library, using a yeast two-hybrid screening method. Following screening of 1×10^7 colonies from the human HeLa cDNA library, we isolated positive clones that appeared to specifically interact with testisin. As an additional check of the positive clones, qualitative blue-white screening with X-gal was performed (Fig. 1A). DNA sequencing and basic alignment searches of the NCBI database revealed that one positive clone corresponded to maspin, which was previously reported as a tumor suppressor molecule in many different cancers [6–8]. To confirm the specific interaction between maspin and testisin in mammalian cells, co-immunoprecipitation experiments were performed with proteins extracted from 293 cells. As shown in Fig. 1B, testisin was detected by Western blot analysis after co-precipitation with anti-maspin antibody, and vice versa. Because testisin is reported to be a GPI-anchored serine protease [3], endogenously expressed testisin may be modified by post-translational events. Therefore, to confirm that endogenous maspin directly interacts with endogenous testisin under conditions where both proteins are naturally expressed, co-immunoprecipitation experiments were performed using proteins extracted from HeLa cells. As shown in Fig. 1C, testisin was detected by Western blot analysis after co-precipitation with anti-maspin antibody. In control experiments using purified preimmune IgG, neither maspin nor testisin was precipitated.

To detect the direct binding of maspin and testisin, we showed that in vitro translated testisin was pulled down with recombinant GST-maspin (Fig. 1D, left) and in vitro translated maspin was pulled down with recombinant GST-testisin (Fig. 1D, right). To examine whether maspin associates with testisin in vivo, localization of maspin and testisin was observed under a confocal microscope. As shown in Fig. 1E, maspin and testisin proteins appeared to colocalize in cytoplasm, indicating that the putative interaction of maspin with testisin may take place in the cytoplasm.

3.2. Testisin blocks maspin-induced activation of capase-3

To examine the possible roles of maspin and testisin in human cervical cancer, we first evaluated several cervical cancer cell lines for mRNA expression of maspin and testisin. RT-PCR analysis demonstrated that maspin and testisin mRNA was expressed in HeLa and MS-751, but not in CaSki cells (Fig. 2A). Based on the previous findings that maspin sensitizes cancer cells to apoptosis [16] and testisin decreases apoptosis [4], we tested whether testisin might influence maspin-mediated increase in caspase-3 activity and cell death. To test for the possible involvement of testisin in modulating maspin-mediated apoptosis, we examined whether down-regulation of testisin or maspin modulated caspase-3 activity using a siRNA knockdown strategy in HeLa and MS-751 cells. As shown in Fig. 2B,

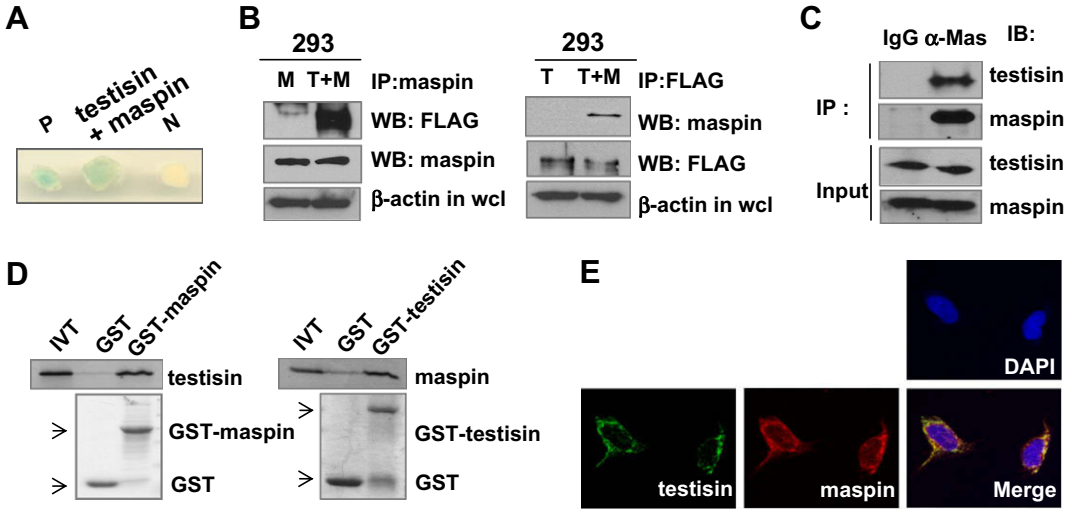

Fig. 1. Testisin interacts with maspin in vitro and in vivo. (A) A yeast two-hybrid system was used to analyze the interaction between testisin and maspin. Maspin and testisin were respectively expressed as fusion proteins in yeast, and transformants were streaked on S.D. medium containing X-gal. P, positive control; N, negative control. (B) Co-immunoprecipitation of maspin with testisin. (left) Total cell lysates from testisin-transfected 293 cells were immunoprecipitated with anti-FLAG antibody, and the presence of maspin in the immunoprecipitates was detected by Western blotting using anti-maspin antibody. (right) Total cell lysates from maspin-transfected 293 cells were immunoprecipitated with anti-maspin antibody and the presence of testisin in the immunoprecipitates was detected by Western blotting using anti-FLAG antibody. (C) Co-immunoprecipitation of endogenous maspin with testisin. Total cell lysates from HeLa cells were immunoprecipitated with anti-maspin antibody, and the presence of testisin in the immunoprecipitates was detected by Western blotting using anti-testisin antibody. (D) (left) Binding of GST-fused recombinant maspin to full-length ^{35}S-testisin. In vitro translated ^{35}S-testisin was pulled down with GST or GST/maspin fusion proteins. (right) Binding of GST-fused recombinant testisin to full-length ^{35}S-maspin. In vitro translated ^{35}S-maspin was pulled down with GST or GST/testisin fusion proteins. Autoradiography after SDS–PAGE resolution shows the in vitro interaction between maspin and testisin. (bottom) Coomassie stained GST fusion proteins from the same gel are aligned to show protein levels. (E) Cytoplasmic co-localization of maspin and testisin. Cells were fixed and stained with DAPI, and the localization of the proteins was examined by confocal microscopy. Merged image of testisin (green), maspin (red) and DAPI-stained DNA (blue) clearly shows co-localization of maspin with testisin.

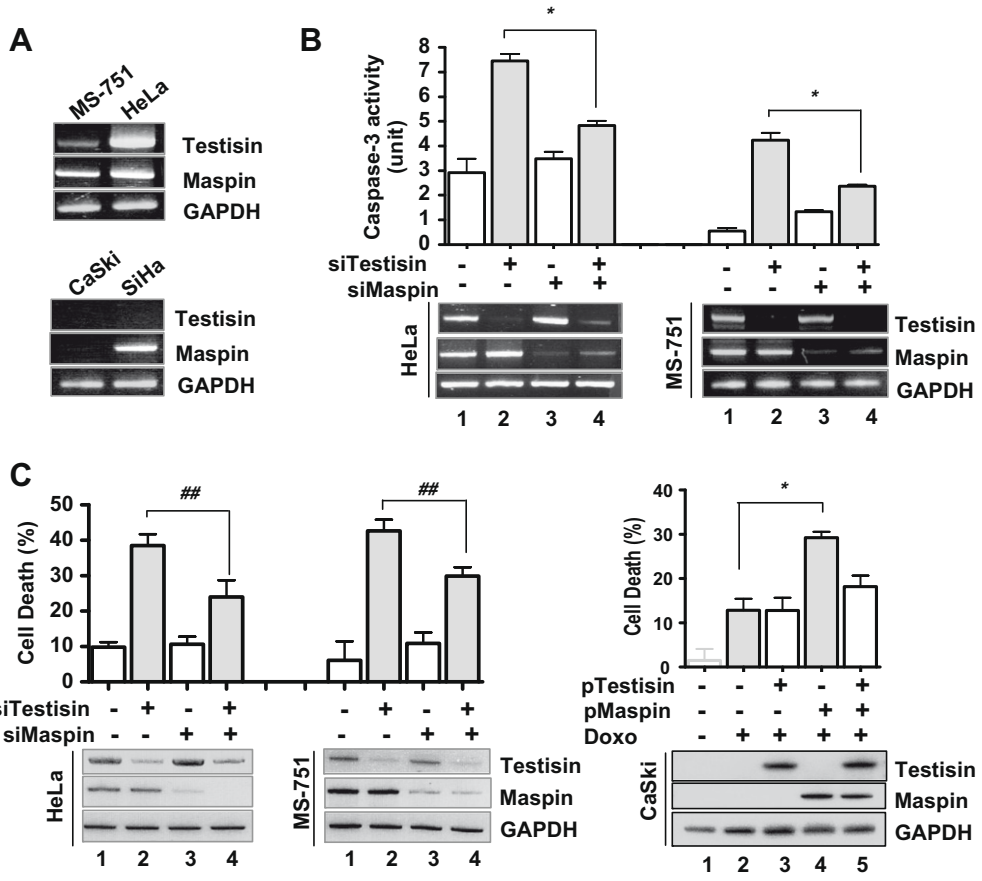

Fig. 2. Testisin prevents maspin-induced increases of caspase-3 activity and cell death. (A) Expression of testisin and maspin in cervical cancer cell lines. (B) Expression of maspin increases caspase-3 activity in the absence of testisin. HeLa and MS-751 cells (1×10^5/well in six-well plates) were transfected with the indicated siRNA or non-specific control siRNA (30 nM). Three days later, the cells were harvested and evaluated for caspase-3 activity (unit). (C) Expression of maspin increases cell death in the absence of testisin. HeLa and MS-751 cells (1×10^5/well in six-well plates) were transfected with siRNA (30 nM). Three days later, the cells were harvested and evaluated for cell death by trypan blue staining. CaSki cells (1×10^5/well in six-well plates) were transfected with the indicated plasmids. Two days later, the cells were treated with doxorubicin (1 μM) for 24 h. The cells were then stained with trypan blue, and the percentage of cells undergoing cell death was scored. Cells were evaluated for testisin and maspin levels by RT-PCR analysis. Data represent the mean ± S.D. of three independent experiments (P values, t-test; bars, S.D.). $^{*}P < 0.001$, $^{**}P < 0.005$, $^{\#}P < 0.01$, $^{\#\#}P < 0.02$.

maspin increased caspase-3 activity in testisin-depleted HeLa and MS-751 cells (lane 3 vs 5, $P < 0.001$). However, in the presence of testisin, maspin did not increase caspase-3 activity (lane 2 vs 4).

3.3. Testisin targets and inactivates maspin in cell death

As testisin blocked maspin-mediated activation of caspase-3, we further tested whether testisin might prevent the maspin-induced cell death. As shown in Fig. 2C, knockdown of maspin decreased cell death of testisin-depleted HeLa and MS-751 cells (lane 2 vs 4, $P < 0.02$). However, without testisin knockdown, maspin knockdown did not affect cell death (lane 1 vs 3, $P > 0.05$). To further confirm the inactivation of maspin by testisin, we performed the experiment using CaSki cells, which do not normally express either testisin or maspin. Expression of recombinant maspin increased cell death in CaSki cells (lane 2 vs 4, $P < 0.001$), while co-expression of recombinant testisin blocked maspin-induced cell death in this system (lane 3 vs 5, $P > 0.05$). In the absence of maspin, recombinant testisin alone did not significantly affect cell death (lane 2 vs 3, $P > 0.05$).

3.4. Testisin knockdown alleviates the doxorubicin resistance of HeLa cells

To determine whether testisin influences drug resistance, we examined the effects of doxorubicin on cell survival of HeLa cells transiently transfected with siTestsin. The knockdown of testisin in these cells was confirmed by RT-PCR analysis (Fig. 3A). Testisin knockdown significantly increased the cell death of doxorubicin treated HeLa cells (Fig. 3B). As shown in Fig. 3C, the median inhibitory concentration (IC_{50}) of doxorubicin was 2.30 μM in control siRNA-transfected HeLa cells, whereas that of siTestisin-transfected HeLa cells was 0.54 μM ($P = 0.016$). These findings suggest that knockdown or inactivation of testisin may sensitize cervical cancer cells to doxorubicin.

3.5. Testisin inactivates maspin-induced suppression of cancer cell invasiveness

As testisin inhibited maspin-mediated cell death, we further tested whether testisin might affect the maspin-mediated suppression of invasion in vitro. As shown in Fig. 4A, knockdown of maspin increased the invasiveness of HeLa and MS-751 (lane 1 vs 3, $P < 0.005$; lane 2 vs 4, $P < 0.02$), even in the presence of testisin, while knockdown of testisin decreased invasion in HeLa and MS-751 cells regardless of maspin expression (lane 1 vs 2, $P < 0.004$; lane 3 vs 4, $P < 0.02$).

As an additional validation, we performed similar experiments using CaSki cells, which do not endogenously express testisin or maspin (Fig. 4B). Invasion by CaSki cells was increased by testisin expression (lane 1 vs 2, $P < 0.001$); this effect was decreased by co-expression of maspin, but not to control levels (lane 1 vs 3,

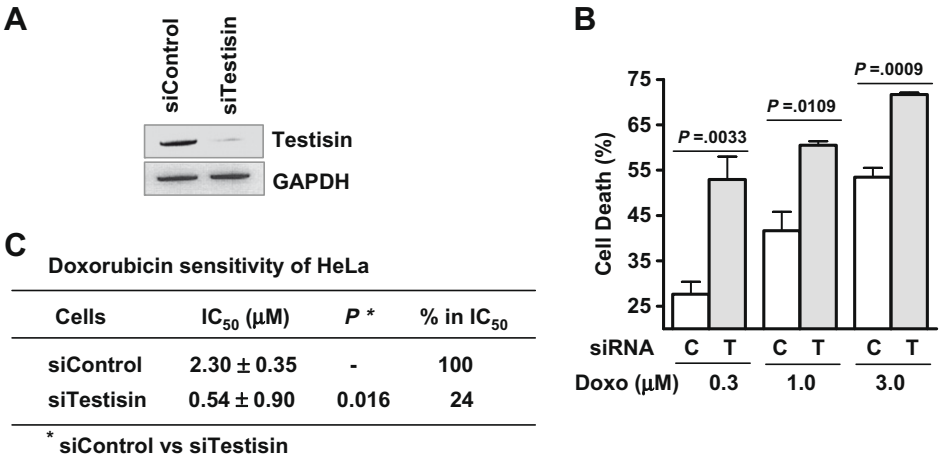

Fig. 3. Testisin knockdown increases drug sensitivity of HeLa cells. (A) HeLa cells were transiently transfected with siControl or siTestisin for siRNA-mediated knockdown of testisin expression. RT-PCR analysis shows that testisin expression was efficiently reduced after transfection of siTestisin (30 nM), whereas control GAPDH expression was unaffected. (B) Cells (1×10^5/well in six-well plates) were transiently transfected with siRNA and incubated for three days, and then treated with indicated doses of doxorubicin for 24 h, followed by trypan blue staining for cell death evaluation. Data represent the mean ± S.D. of three independent experiments (*P* values, *t*-test; bars, S.D.). (C) Cells (1×10^4/well in 96-well plates) were treated with increasing doses of doxorubicin for 24 h, followed by MTT assay. Data shown are representative of three independent experiments, and the means and standard deviations from the same experiment are given in the table.

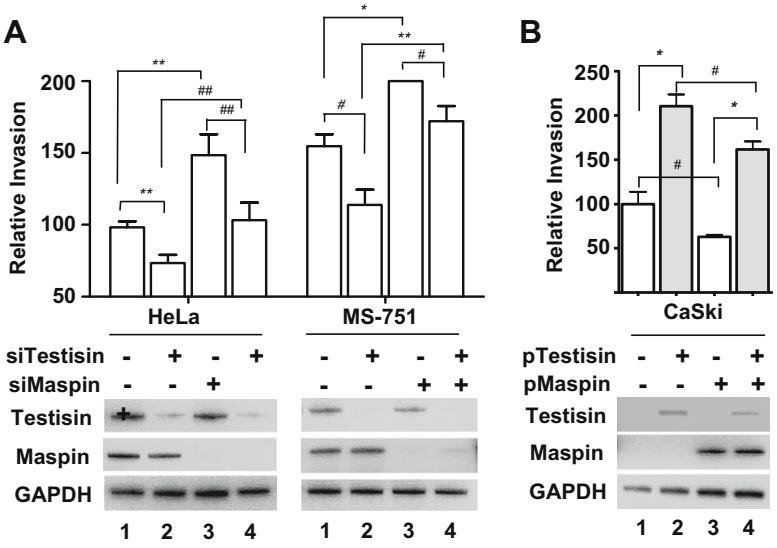

Fig. 4. The expression of testisin and maspin determines the invasiveness of cervical cancer cells. (A) Knockdown of testisin and maspin modulates the invasiveness of HeLa and MS-751 cells. Cells (1×10^5 cells/well in six-well plates) were transfected with the indicated siRNA or non-specific control siRNA (30 nM). After two days, the cells were starved for 24 h and then plated onto Matrigel-coated membranes (5×10^4/well in 96-well plates; after 24 h, invasive cells were counted. (B) Expression of testisin and maspin modulates the invasion of CaSki cells. Cells (1×10^5cells/well in six-well plates) were transiently transfected with the indicated plasmids or control vector. Two days later, the cells were starved for 24 h and then plated for the above-described invasion analysis. Expression of testisin or maspin was examined by RT-PCR, with GAPDH used as a control. Data represent the mean ± S.D. of three independent experiments (*P* values, *t*-test; bars, S.D.). $^{*}P < 0.001$, $^{**}P < 0.005$, $^{\#}P < 0.01$, $^{\#\#}P < 0.02$.

$P < 0.01$). Furthermore, maspin expression decreased the invasiveness of CaSki cells (lane 2 vs 4, $P < 0.01$), but this invasiveness was restored by co-expression of testisin (lane 3 vs 4, $P < 0.001$). These results indicate that testisin and maspin may oppose each other in the control of invasion.

3.6. Expression of testisin is increased in human cervical cancer

Despite some evidence that testisin may be correlated with cell transformation, testisin gene expression seems to be restricted to male germ cells, and is lost in germ cell tumors. Therefore, to exclude possible cell line artifacts, we further validated the presence of testisin in human cervical cancer specimens. As shown in Fig. 5,

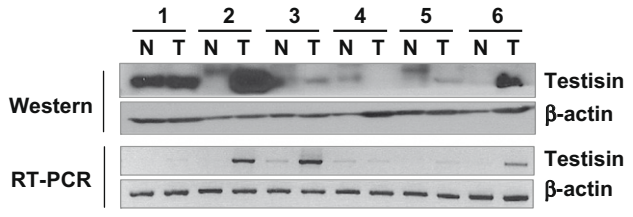

Fig. 5. Testisin level is increased in human cervical tumors. RT-PCR and Western blot analysis of testisin in human cervical tissues. Freshly frozen specimens of cervical tumors (T) show increased testisin expression compared with corresponding normal tissues (N). β-Actin was used as a loading control.

the expression level of testisin was found to be increased in four of six tumor samples, compared to normal controls, by both RT-PCR and Western blot analysis.

4. Discussion

In order for tumor to progress, they must bypass the program leading to cell death and enter one promoting metastasis, most likely via multiple interactions of intracellular molecules. Therefore, identification of interacting molecules responsible for controlling apoptosis and metastasis may facilitate the development of new cancer treatment options. Testisin and maspin have been implicated in malignant transformation as an inhibitor and a promoter, respectively. However, this is the first report of an interaction between these two proteins.

Serine proteases are engaged in critical intracellular functions including apoptosis and tumor growth [17–19]. Recent evidence also suggests that proteases from cancer cells may function as modulators of tumor–stroma interactions [20,21]. Testisin is a gelatin-hydrolyzing serine protease, which has a distinct carboxy-terminal peptide modified with a glycosylphosphatidylinositol anchor to enable localization on the cell surface [4]. Testisin mRNA expression has been found in several tumor cell lines of different cellular origins, including ovarian cancer, cervical cancer, melanoma, and lymphoma [1–4]. Although testisin induces colony formation and promotes malignant transformation in cancer cells [4], the action mechanism or physiologic substrates for testisin have not been previously determined. This is the first study to examine how testisin might promote cervical carcinogenesis. We used a yeast two-hybrid system to identify maspin as a novel target of testisin.

Maspin was found to be a Bax-activating protein, thereby contributing to apoptosis and tumor suppression [16]. Maspin has been found to interact with diverse intra- and extracellular molecules, such as tissue-type plasminogen activator [22], pro–urokinase type plasminogen activator [23], IFN responsive factor 6 [24], collagen type I [25], glutathione S-transferase [26], and histone deacetylase 1 [27]. Considering that testisin is a serine protease [1] that is frequently amplified in human cancers, it is not surprising that testisin interact with maspin, which has a high sequence homology with serine protease inhibitors [5]. Despite having high sequence homology to the serpin family protease inhibitor, maspin is known as a non-inhibitory serpin [28,29]. Although we do not have sufficient evidence to conclude whether proteolytic activity of testisin is critical for inhibition of maspin, Western blot data in Figs. 2 and 4 clearly show that maspin is not a proteolytic substrate of testisin.

We examined the testisin and maspin interaction and its consequences in malignant cervical cancer cells. Knockdown of maspin decreased the cell death in testisin-depleted cervical cancer cells but not in cells expressing endogenous levels of testisin. Additional experiments revealed that expression of testisin increased the survival of CaSki cells under doxorubicin-mediated DNA damage-inducing conditions, but only in the presence of maspin, suggesting that testisin may negatively regulate maspin-induced cell death in human cancer cells. Using siRNA specific for testisin, we also showed that testisin knockdown decreased doxorubicin resistance of HeLa cells.

Previous studies have shown that knockdown of testisin expression leads to increased apoptosis [4]. However, we herein found that this effect requires the presence of maspin, as evidenced by the prevention of cell death by recombinant testisin expression only in the presence of maspin in CaSki cells. Out results may provide the basis for better understanding of the role of testisin and maspin in cancer progression. Furthermore, this emerging testisin function may provide a mechanistic explanation for the poor prognosis of some maspin-overexpressing cancers [30,31]. Therefore, further studies will be necessary to clarify the contribution of testisin and maspin in other cancers.

In conclusion, we herein show for the first time that the tumor suppressor maspin is a target of testisin. On the basis of our results, we conclude that testisin not only blocks the maspin-mediated cell death pathway, but also increases the invasion of cells, thereby contributing to carcinogenesis.

Acknowledgements

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