Mechanism of apoptotic cell death of human gastric carcinoma cells mediated by transforming growth factor β

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Human gastric carcinoma cell line HSC-39 has been shown to undergo apoptotic cell death in response to treatment with transforming growth factor β_1 (TGF- β_1). To understand better the cell death mechanism in this TGF- β_1 -mediated apoptosis, we investigated the effect of the expression of TGF- β -stimulated clone 22 (TSC-22) on cell death events. TGF- β_1 induced TSC-22 gene expression in HSC-39 cells only when the cells had previously been adapted to the serum-free culture conditions required to undergo TGF- β_1 -mediated apoptosis. HSC-39 cells transfected with a TSC-22 expression vector showed a significant decrease in cell viability compared with those transfected with a control vector. The cellular events characteristic of apoptosis, chromatin condensation and DNA fragmentation were observed only in

cells transfected with a TSC-22 expression vector. On immunostaining of the transfected cells, almost every cell that expressed TSC-22 tagged with influenza virus haemagglutinin exhibited the morphology of an apoptotic cell. Partial protection from the cell death effect of TGF- β_1 on HSC-39 cells was observed when cells were treated with acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartyl-L-al (Ac-DEVD-CHO, an inhibitor specific for CPP32-type protease). Protection against cell death by the transfection of a TSC-22 expression vector was also offered by Ac-DEVD-CHO addition. These results suggest that TSC-22 elicits the apoptotic cell death of human gastric carcinoma cells through the activation of CPP32-like protease and mediates the TGF- β_1 signalling pathway to apoptosis.

INTRODUCTION

Transforming growth factor β (TGF- β) has been shown to be a multifunctional growth regulator that either stimulates the proliferation of mesenchymal cells or inhibits the growth of epithelial cells, such as those of colon carcinoma [1], breast carcinoma [2] and gastric carcinoma [3]. The inhibitory effect of TGF- β is fully reversible [4]. It has been shown that, in addition to growth regulation, TGF- β can elicit apoptotic cell death in both normal and malignant cells under certain conditions. TGF- β has been found to induce apoptosis in endometrial cells [5], normal and transformed hepatocytes [6,7], acute myelogenous leukaemia cells [8] and a number of other cell types. However, it is still not clear why TGF- β causes reversible growth arrest in some cells but other cells undergo apoptosis. Apoptosis is a cell death process found in eukaryotes. All multicellular organisms have death mechanisms to kill their own cells for development, homeostasis and aging. The death of cells undergoing apoptosis is preceded by morphological changes and the fragmentation of chromatin by a specific Ca^{2+} -regulated nuclease at the linker region between nucleosomes [9]. The recently established human gastric carcinoma cell line HSC-39 was shown to undergo apoptosis when the cells were treated with TGF- β_1 under serum-free culture conditions [10]. This cell line requires new protein synthesis to undergo apoptosis, but further mechanisms underlying this TGF- β_1 -mediated apoptosis remain unknown. To investigate the factors involved we focused on TGF- β -stimulated clone 22 (TSC-22), originally identified as a clone greatly induced

by TGF- β_1 in mouse osteoblastoma cells [11] and speculated to be a transcriptional regulator owing to its possession of the leucine zipper motif and its nuclear localization. We found that TSC-22 gene expression is also induced by TGF- β_1 in HSC-39 cells. In this study we examined the effect of TSC-22 expression in HSC-39 cells and found that TGF- β_1 -mediated apoptosis is correlated with the induction of TSC-22 and that the ectopic expression of TSC-22 in HSC-39 cells leads to apoptotic cell events. We also refer to the possible involvement of CPP32-like protease in TGF- β_1 -mediated apoptosis.

EXPERIMENTAL

Materials

Recombinant human TGF- β_1 was obtained from Wako Pure Chemicals (Osaka, Japan). Hoechst dye 33258 was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-L-al (Ac-YVAD-CHO) and acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartyl-L-al (Ac-DEVD-CHO) were obtained from Peptide Institute (Osaka, Japan). Human glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA probe was obtained from Clontech (Palo Alto, CA, U.S.A.). DMRIE-C reagent consisting of a 1:1 (mol/mol) liposome formulation of the cationic lipid 1,2-dimyristyl-oxypropyl-3-dimethylhydroxyethyl ammonium bromide (DMRIE) and cholesterol was obtained from Gibco BRL (Gaithersburg, MD, U.S.A.). Monoclonal antibody against

Abbreviations used: Ac-DEVD-CHO, acetyl-L-aspartyl-L-glutamyl-L-valyl-L-aspartyl-L-al; Ac-YVAD-CHO, acetyl-L-tyrosyl-L-valyl-L-alanyl-L-aspartyl-L-al; CDM, chemically defined medium; DMRIE, 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl ammonium bromide; FCS, fetal calf serum; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HA, influenza virus haemagglutinin epitope; ICE, interleukin 1 β -converting enzyme; α -MEM, α -minimal essential medium; TGF- β , transforming growth factor β ; TSC-22, TGF- β -stimulated clone 22.

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influenza virus haemagglutinin epitope (HA) and FluoroLink Cy3-labelled goat anti mouse IgG antibody were obtained from Boehringer Mannheim GmbH (Mannheim, Germany) and Amersham International plc (Little Chalfont, Bucks., U.K.) respectively.

Cell line and culture conditions

HSC-39 human gastric carcinoma cells were grown in α -minimal essential medium (α -MEM) supplemented with 10% (v/v) fetal calf serum (FCS) as previously described [10]. To analyse the effect of TGF- β_1 , cells had previously been adapted to serum-free chemically defined medium (CDM), which consisted of Dulbecco's modified Eagle's medium/Ham's F12 medium (1:1) supplemented with 0.05% BSA, for at least 2 weeks; recombinant human TGF- β_1 was then added in the same serum-free CDM at a concentration of 2 ng/ml.

Northern blot analysis

Total cytoplasmic RNA was prepared from cultured cells in accordance with the method described in [12]. Cytoplasmic RNA (5 μ g per lane) was electrophoretically separated in a denatured 1.4% (w/v) agarose gel and then transferred to a Hybond-N nylon membrane (Amersham). The membrane was hybridized with a 32 P-labelled human TSC-22 cDNA probe (see Figure 1) or a human CPP32 cDNA probe (see Figure 4B) overnight at 65 °C in 0.5 M sodium phosphate (pH 7.5)/10 mg/ml BSA/1 mM EDTA/7% (w/v) SDS, followed by washing twice for 30 min at 50 °C in $2\times$ SSC ($1\times$ SSC is here defined as 0.16 M NaCl/0.016 M sodium citrate)/0.1% SDS. The membrane was sequentially hybridized with a 32 P-labelled human G3PDH probe as control.

Construction of expression vectors and transfection

Rat TSC-22 full-length cDNA was subcloned into the pEF-BOS expression vector [13] at an *Xba*I site under the control of elongation factor promoter, to obtain pEF-TSC22. To construct HA-tagged TSC-22 expression vector (pCG-HATSC22), rat TSC-22 cDNA was subcloned into pCG-N-BL vector [14] at an *Sma*I site (the amino acid sequence around the fusion region was confirmed by DNA sequencing as HA1 epitope-Ser-Arg-Thr-Ser-Gly-Ser-Pro-Asn-Ser-rat TSC-22). HSC-39 cells (2×10^6 cells) were transfected with plasmid DNA (2 μ g) with DMRIE-C reagent essentially in accordance with the manufacturer's protocol. Cells were treated with DNA-DMRIE mixture in OPTI MEM I reduced-serum medium (Gibco BRL) for 5 h, after which the medium was changed to serum-free CDM or α -MEM/10% (v/v) FCS.

Apoptosis assay

For the cell viability assay, cell suspensions were scored for numbers of viable and non-viable cells by Trypan Blue dye exclusion, and quantification was performed using a haemocytometer. In each sample a minimum of 500 cells were counted and cell viability was expressed as a percentage relative to untreated or control transfected cells. For morphological evaluation, cells were fixed on glass slides with 4% (v/v) formaldehyde in PBS, pH 7.4, for 5 min at room temperature, and stained with Hoechst dye 33258 at 0.1 μ g/ml in PBS. Nuclear morphology was observed with a fluorescence microscope. For DNA fragmentation analysis, cell pellets were lysed in 50 mM Tris/Cl (pH 8)/10 mM EDTA/0.5% SDS, treated with DNase-free RNase A (0.2 mg/ml) for 30 min at 50 °C, and then treated with proteinase K (0.2 mg/ml) for 1 h at 50 °C.

DNA was extracted once with phenol and once with phenol/chloroform (1:1, v/v), then precipitated with ethanol. Recovered DNA (10 μ g each) was then subjected to electrophoresis in a 2% (w/v) agarose gel and detected by ethidium bromide staining.

Immunostaining

At 48 h after transfection with pCG-HATSC22 or pCG-N-BL empty vector for control, HSC-39 cells were washed twice with PBS, and collected on glass slides with a cytospin system (Tomy Seiko, Tokyo, Japan). Cells were then fixed with acetone for 5 min at -20 °C and air-dried. After washing twice with PBS, fixed cells were incubated with anti-HA monoclonal antibody (diluted 1:500) for 2 h at room temperature, washed extensively and then incubated with FluoroLink Cy3-labelled goat anti-mouse IgG antibody (diluted 1:500). After washing with PBS, cells were detected by using a fluorescence microscope.

RESULTS

Induction of TSC-22 mRNA in HSC-39 cells by TGF- β_1

Human gastric carcinoma HSC-39 cells previously adapted to serum-free CDM were treated with TGF- β_1 , and cytoplasmic RNA was prepared for Northern blot analysis of TSC-22 gene expression. Significant induction of TSC-22 mRNA was observed after 2 h of treatment with TGF- β_1 ; the increase lasted for at least 10 h, whereas G3PDH mRNA did not show any change (Figure 1). TSC-22 gene expression was low in non-treated cells or in cells cultured in α -MEM supplemented with 10% (v/v) FCS.

Apoptosis of HSC-39 cells transfected with TSC-22 expression vector

HSC-39 cells were transfected with a TSC-22 expression vector (pEF-TSC22) or a control vector (pEF-BOS); cell viability was

Figure 1 Northern blot analysis of TSC-22 mRNA level in HSC-39 cells after TGF- β_1 treatment

Cells were cultured in α -MEM/10% (v/v) FCS (lane 1) or adapted to serum-free CDM (lane 2) for 2 weeks. Cells previously adapted to serum-free CDM conditions were treated with TGF- β_1 (2 ng/ml) for 2, 4, 12 or 24 h. Next, cytoplasmic RNA was prepared, separated in a denaturing gel (5 μ g per lane) for Northern blot and sequentially hybridized with TSC-22 and G3PDH probes.

Table 1 Effects of treatment with TGF- β_1 and transfection with pEF-TSC22 on viability of HSC-39 cells

Cells adapted to serum-free CDM culture conditions were treated with TGF- β_1 (2 ng/ml) or transfected with pEF-TSC22. After 48 h of incubation, cell viability was determined and expressed as a percentage relative to untreated or control transfected cells. The effect of pEF-TSC22 transfection on cells not adapted to serum-free conditions was also examined. Results from three independent experiments are shown as means $\pm$ S.D. *Significantly different from control ($P < 0.01$), by Student's t test.

Culture conditions	Treatment	Cell viability (%)
Adapted to serum-free CDM culture conditions	None (control)	100.0 $\pm$ 1.8
	2 ng/ml TGF- β_1	35.2 $\pm$ 4.8*
	Transfected with pEF-BOS (control)	100.0 $\pm$ 9.3
	Transfected with pEF-TSC22	28.1 $\pm$ 3.1*
Not adapted (α -MEM/10% FCS)	Transfected with pEF-BOS (control)	100.0 $\pm$ 7.1
	Transfected with pEF-TSC22	61.0 $\pm$ 6.0*

measured 48 h later. When HSC-39 cells had previously been adapted to serum-free CDM culture conditions to acquire sensitivity to TGF- β_1 -mediated apoptosis, pEF-TSC22 transfection significantly decreased cell viability compared with control pEF-BOS transfection to an extent similar to that caused by TGF- β_1 treatment (Table 1). Although HSC-39 cells did not exhibit any apoptotic phenomena under culture conditions of α -MEM/10% (v/v), FCS pEF-TSC22 transfection of cells cultured in this serum-containing medium caused a significant decrease in cell viability as observed in the serum-free medium.

To investigate whether the decrease in cell viability in HSC-39 cells transfected with pEF-TSC22 was due to apoptotic cell death, as with TGF- β_1 treatment, we next examined the morphological change of the cell nucleus by using Hoechst 33258 fluorochrome staining. HSC-39 cells cultured in α -MEM/10%

(v/v) FCS exhibited chromatin condensation, characteristic of apoptosis, at 48 h after transfection with pEF-TSC22, whereas cells transfected with pEF-BOS did not show such a morphological change in the nuclei (Figure 2A). A similar morphological change was observed in cells previously adapted to serum-free CDM and transfected with pEF-TSC22 (results not shown). Apoptotic cell death of HSC-39 cells by pEF-TSC22 transfection was also identified by DNA fragmentation analysis. Cellular DNA prepared from HSC-39 cells at 48 h after transfection with pEF-TSC22 exhibited a ladder pattern, also characteristic of apoptosis; no such pattern was observed in the DNA preparation from cells transfected with pEF-BOS (Figure 2B). These results strongly suggest that the ectopic expression of TSC-22 in HSC-39 cells caused apoptotic cell death even under conditions not sensitive to TGF- β_1 -mediated apoptosis.

Correlation of TSC-22 expression with apoptosis of HSC-39 cells

To examine the correlation between TSC-22 expression and apoptotic cell morphology, an HA-tagged TSC-22 expression vector (pCG-HATSC22) was constructed and transfected into HSC-39 cells. At 48 h after transfection, cells were subjected to immunostaining with anti-HA monoclonal antibody. Figure 3 shows that almost every cell positive to anti-HA antibody, and therefore expressing TSC-22, exhibited the abnormal cell morphology characteristic of apoptosis, indicating a one-to-one correspondence of TSC-22 expression with apoptotic cell death.

Involvement of CPP32-like protease activity in both TGF- β_1 -mediated and TSC-22-induced apoptosis

Much evidence supports the involvement of cysteine proteases related to interleukin 1 β -converting enzyme (ICE) in several apoptosis systems [15,16]. For example, ICE-like protease and CPP32-like protease are sequentially activated during Fas-mediated apoptosis [17]. We next examined the effects of specific

Figure 2 Characterization of cell death of HSC-39 cells caused by pEF-TSC22 transfection

(A) HSC-39 cells cultured in α -MEM/10% (v/v) FCS were transfected with pEF-TSC22 or pEF-BOS. After 48 h, cells were fixed and stained with Hoechst 33258 fluorochrome. (B) DNA was prepared from cells transfected as in (A) and fragmentation was analysed on a 2% (w/v) agarose gel.

Figure 3 Correlation of ectopic TSC-22 expression with apoptotic cell morphology

HSC-39 cells cultured in α -MEM/10% FCS were transfected with an HA-tagged TSC-22 expression vector (pCG-HATSC22). After 48 h, transfected cells were examined by immunostaining with anti-HA monoclonal antibody and Cy3-labelled secondary antibody. (A) Saline was added instead of anti-HA primary antibody. (B) Immunostaining of cells expressing HA-tagged TSC-22; cells positive to anti-HA antibody are indicated by arrowheads. (C) The same visual field as in (B) observed under a phase-contrast microscope. Cells corresponding to HA-positives in (B) are also indicated by arrowheads.

Table 2 Involvement of CPP32-like protease activity in TGF- β_1 -mediated or TSC-22-induced apoptosis of HSC-39 cells

Cells previously adapted to serum-free conditions were incubated with 0.5 mM protease inhibitors for 3 h, after which TGF- β_1 (2 ng/ml) was added. At the same time, cells cultured in α -MEM/10% FCS were transfected with pEF-TSC22 (or pEF-BOS as control). After 5 h the medium was changed to α -MEM/10% FCS containing 0.5 mM inhibitors. Cell viability was measured 24 h later and is expressed as a percentage relative to control cells. Results from three independent experiments are shown as means $\pm$ S.D. *Significantly different from control ($P < 0.01$) by Student's *t* test; n.s., not significant.

Culture conditions	Treatment	Inhibitor	Cell viability (% of control)
Adapted to serum-free CDM culture conditions	None 2 ng/ml TGF- β_1	None	100.0 $\pm$ 8.0
		None (control)	39.0 $\pm$ 3.8
		Ac-YVAD-CHO	43.2 $\pm$ 1.6 n.s.
		Ac-DEVD-CHO	64.8 $\pm$ 6.0*
Not adapted (α -MEM/10% FCS)	Transfected with pEF-BOS Transfected with pEF-TSC22	None	100.0 $\pm$ 8.0
		None (control)	69.5 $\pm$ 4.8
		Ac-YVAD-CHO	75.2 $\pm$ 5.4 n.s.
		Ac-DEVD-CHO	91.6 $\pm$ 3.0*

inhibitors for such proteases on TGF- β_1 -mediated apoptosis of HSC-39 cells. Peptide inhibitors specific to CPP32-type protease (Ac-DEVD-CHO) or to ICE-type protease (Ac-YVAD-CHO)

Figure 4 Involvement of CPP32-like protease activity in TGF- β_1 -mediated or TSC-22-induced apoptosis of HSC-39 cells

(A) HSC-39 cells cultured in α -MEM/10% FCS were transfected with pEF-TSC22 (or pEF-BOS for control) mixed with DMR1E-C reagent in OPTI MEM for 5 h, after which the medium was changed to α -MEM/10% FCS containing 0.5 mM Ac-DEVD-CHO or Ac-YVAD-CHO. After 24 h, DNA was prepared from the cells and subjected to electrophoresis. The DNA fragmentation pattern was revealed by ethidium bromide staining. Lane 1, DNA from pEF-BOS-transfected cells without addition of inhibitor; lane 2, DNA from pEF-TSC22-transfected cells without addition of inhibitor; lane 3, DNA from pEF-TSC22-transfected cells with addition of Ac-YVAD-CHO; lane 4, DNA from pEF-TSC22-transfected cells with addition of Ac-DEVD-CHO. (B) Cytoplasmic RNA was prepared from cells cultured in α -MEM/10% FCS (lane 1), cells adapted for 2 weeks to serum-free CDM (lane 2) or cells treated with TGF- β_1 (2 ng/ml) for 24 h after serum-free adaptation (lane 3) and subjected to Northern blot analysis with a human CPP32 cDNA probe. The same nylon membrane was also hybridized with a human G3PDH probe as control.

[18] were added at 0.5 mM to HSC-39 cells previously adapted to serum-free culture conditions. After 3 h of incubation, TGF- β_1 (2 ng/ml) was added to cells and cell viability was measured at 24 h after TGF- β_1 addition (viability of HSC-39 cells was not affected by these inhibitors at 0.5 mM; results not shown). As

can be seen from Table 2, partial protection against TGF- β_1 -mediated cell death was offered by Ac-DEVD-CHO addition but not by Ac-YVAD-CHO addition, suggesting that CPP32-like protease activity is at least partly required for this TGF- β_1 -mediated apoptotic process and that the activation of CPP32-like protease is independent of ICE-like protease activity. The effects of inhibitors on TSC-22-induced cell death were also examined. HSC-39 cells cultured in α -MEM/10% (v/v) FCS were incubated with pEF-TSC22 and DMR1E-C reagent in OPTI MEM for transfection. After 5 h of incubation, the medium was changed to α -MEM/10% (v/v) FCS containing 0.5 mM Ac-DEVD-CHO or Ac-YVAD-CHO, and 24 h later cell viability and DNA fragmentation were measured. Similarly to TGF- β_1 -mediated cell death, TSC-22-induced decrease in cell viability was also protected by Ac-DEVD-CHO addition but not by Ac-YVAD-CHO addition (Table 2). Protection against DNA fragmentation caused by pEF-TSC22 transfection was also offered only by the addition of Ac-DEVD-CHO (Figure 4A). These results strongly suggest that activation of CPP32-like protease through a pathway independent of ICE-like activity is involved in the apoptotic process induced by TSC-22 expression. Activation of ICE-related proteases has been reported to be due to post-transcriptional modification [17]. No significant alteration in CPP32 mRNA level was observed in Northern analysis of HSC-39 cells before and after TGF- β_1 treatment (Figure 4B); the activation of CPP32-like protease therefore seems also to occur at the post-transcriptional level in this apoptosis system.

DISCUSSION

TGF- β_1 elicits apoptosis in human gastric carcinoma HSC-39 cells under serum-free culture conditions [10]. Partial protection against this TGF- β_1 -mediated apoptosis is offered by cycloheximide, but further mechanisms have not yet been clarified. Our observation that the expression of TSC-22 in HSC-39 cells elicited apoptotic cell events suggests a possible mechanism of TGF- β_1 -mediated apoptosis of HSC-39 cells for several reasons. First, TSC-22 is involved in the TGF- β_1 signal transduction pathway in certain cell types [11,19]. Secondly, the expression of TSC-22 was greatly stimulated by TGF- β_1 in HSC-39 cells only when the cells had been adapted to culture conditions undergoing apoptosis. Thirdly, almost every HSC-39 cell expressing HA-tagged TSC-22 showed apoptotic cell morphology on immunostaining. TGF- β_1 could not induce apoptosis when HSC-39 cells were cultured in α -MEM/10% (v/v) FCS, whereas pEF-TSC22 transfection had a significant apoptotic effect on cells even under culture conditions in the presence of serum. The steady-state level of bcl-2 mRNA in HSC-39 cells greatly decreased when the cells were adapted to serum-free CDM, and the level remained low during TGF- β_1 treatment (results not shown). Therefore transcriptional down-regulation of bcl-2 might be the reason for the alteration of the cellular response to TGF- β . It seems possible that overexpression of TSC-22 in HSC-39 cells cultured in serum-containing medium can overcome the negative effect of the bcl-2 product on apoptotic processes.

TSC-22 was originally identified as a clone that was stimulated in mouse osteoblast cells by treatment with TGF- β [11]. It has a putative leucine zipper domain and has been shown to be localized in the cell nucleus. It has therefore been speculated that it is a transcriptional regulator. However, the actual biological function of TSC-22 remains unknown. The results reported here indicate a novel biological role for TSC-22 as an apoptosis mediator. We recently reported observations supporting the function of TSC-22 as a transcriptional regulator [20]. Some

transcription factors such as p53, c-Myc and AP-1, have been shown to have important roles in apoptotic cell death. However, the true targets of those transcription factors in the apoptotic cell death events are still unknown. Recent studies show that p53 down-regulates and up-regulates the expression of bcl-2 and bax respectively [21,22]; however, the actual cell death mechanism is still controversial.

At least some part of the TGF- β_1 -mediated apoptosis of HSC-39 cells requires the activation of CPP32-like protease. TSC-22-induced apoptosis also requires CPP32-like protease activity to a greater extent than TGF- β_1 -mediated apoptosis, judging from the almost complete protection from the cell death effect of pEF-TSC22 transfection by the addition of Ac-DEVD-CHO. Activation of CPP32-like protease in this TGF- β_1 -mediated apoptosis system seems to be through some unknown pathway other than the ICE-mediated pathway as demonstrated in the Fas system [17]. This activation of CPP32-like activity seems to occur post-transcriptionally because there is no alteration of CPP32 mRNA level during the apoptotic process. Alternatively, a protease with CPP32-type substrate specificity other than CPP32 itself might be up-regulated. In any case, TSC-22 seems to be capable of activating CPP32-like protease activity.

The idea that TGF- β_1 -mediated apoptosis of HSC-39 cells involves the stimulation of TSC-22 coincides with the previous observation of the requirement of new protein synthesis for apoptosis to occur. Recently, human hepatoma cell line Hep 3B was also reported to undergo apoptosis in response to TGF- β [23]. The mechanism of the TGF- β_1 -mediated cell death of hepatoma, however, seems somewhat different from that of HSC-39 cells because treatment with cycloheximide had no effect on the TGF- β_1 -mediated hepatoma cell death and no chromatin condensation was observed in that apoptosis system. Therefore it must be examined whether TSC-22 is involved in other TGF- β_1 -mediated apoptosis systems such as hepatoma cells and normal hepatocytes. Another interesting question for future work is whether TSC-22 acts as a transcriptional regulator in TGF- β_1 -mediated apoptosis.

In conclusion, this paper provides the first evidence for TSC-22 eliciting apoptotic cell death through the activation of CPP32-like protease in human gastric carcinoma cells and possibly mediating the apoptotic cell death induced by TGF- β_1 .

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