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BASIC INVESTIGATION

Effects of Saikosaponin-D on syndecan-2, matrix metalloproteinases and tissue inhibitor of metalloproteinases-2 in rats with hepatocellular carcinoma

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Abstract

OBJECTIVE: To investigate effects of Saikosaponin D (SSd) on syndecan-2, matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases-2 (TIMP-2) in livers of rat with hepatocellular carcinoma (HCC).

METHODS: Male SD rats were divided into control (n=10), model (n=20) and SSd (n=20) groups, and model and SSd groups given intragastric 0.2% (w/v) N-diethylnitrosamine to induce HCC. SSd group received 0.03% (w/v) SSd in saline. Liver samples were analysed immunohistochemically for syndecan-2, MMP-2, MMP-13 and TIMP-2 at 16 weeks.

RESULTS: The model group had more malignant nodules than the SSd group; all model-group HCC cells were grade III; SSd-group HCC cells were grades I-II. Controls showed normal hepatic cell phenotypes and no syndecan-2$^+$ staining. Syndecan-2$^+$ staining was greater in the model group (35.2%, P≤0.001) than in controls or the SSd group (16.5%, P ≤ 0.001). The model group had more intense MMP-2$^+$ staining than controls (0.37 vs 0.27, P≤0.01) or the SSd group (0.31 vs 0.37, P≤0.05); and higher MMP-13$^+$ staining (72.55%) than in controls (12.55%, P≤0.001) and SSd group (20.18%, P≤0.01). The model group also had more TIMP-2$^+$ staining (57.2%) than controls (20.9% , P≤0.001) and SSd group (22.7% , P≤0.001). Controls and SSd group showed no difference in TIMP-2$^+$ rates.

CONCLUSION: SSd inhibited HCC development, and downregulated expression of syndecan-2, MMP-2, MMP-13 and TIMP-2 in rat HCC liver tissue.

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Key words: Carcinoma, Hepatocellular; Saikosaponin; Syndecan-2; Matrix metalloproteinases; Tissue inhibitor of metalloproteinase inhibitor-2

INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most common cancer and the third leading cause of cancer-related deaths in the world, causing 500,000 deaths annually, of which approximately half occur in China.[1,2] The development of HCC is a multi-step process including cellular adhesion and the destruction of extracellular matrix (ECM). During this process, different molecules in the ECM and receptors and/or ligands on the surfaces of tumor cells play critical roles.

Syndecans (syndecan-1, -2, -3, and -4) are a family of

heparan sulfate proteoglycans (HSPGs) contain a single transmembrane core protein, with several heparan sulfate (HS) and chondroitin sulfate (CS) side chains attached. Previous studies have shown that syndecans are involved in the development and metastasis of HCC.[3-7] Interestingly, different syndecan isoforms seem to play distinct roles in carcinogenesis and invasion. Syndecan-1expression in human HCC tissue is negatively correlated with histologic dedifferentiation, metastasis, and poor prognosis.[3,5] However, syndecan-4 has been shown to induce human hepatoma growth by activating the cell cycle and inhibiting tumor necrosis factor alpha (TNF-α)-mediated apoptosis.[7] Syndecan-3 expression is also seen in HCC tissue, especially in tumoral stromal vessels,[6] suggesting it may play some roles in tumoral angiogenesis.

The relationship between syndecan-2 and HCC is largely unknown; only a recent cDNA microarray study suggested that syndecan-2 gene expression increases in HCC tissue.[4] However, accumulating evidence indicates that syndecan-2 affects other carcinoma occurrence and invasion such as pancreatic[8] and prostate cancers.[9] It is unclear how syndecan-2 affects cancer cell behavior, dedifferentiation and invasion. However, considering that syndecans localizes on cell surfaces, and its HS side chains can bind to a large variety of ligands in the ECM, including vascular endothelial growth factor (VEGF), an important growth factor involved in HCC[10] angiogenesis and metastasis, it is likely that syndecan-2 influences ligand-induced activation and signaling in cells, consequently inducing cell migration[11] and angiogenesis.[12] Moreover, syndecans bind to many structural ECM molecules such as collagens I, III and V, fibronectin, thrombospondin and tenascin, which provide structural support for the cell – ECM adhesion, and are already known to be involved in HCC invasiveness and progression.[13-16]

Matrix metalloproteinases (MMPs) play critical roles in carcinogenesis, including the destruction of ECM and basement membranes.[17] MMP expression positively correlates with invasion and metastasis of HCC and other malignant tumors.[18,19] Interestingly, MMP-2, MMP-9 and their major inhibitor, the tissue inhibitor of matrix metalloproteinase inhibitor-2 (TIMP-2) apparently influence HCC development and invasion, as their higher expression in HCC tissues correlates more closely to patient outcome than other MMPs and TIMPs.[20-22] The precise mechanism behind this correlation is not very clear, although MMP-2 and MMP-9 are thought to affect ECM degradation, which facilitates invasion and metastasis of tumor cells.[23] Besides MMP-2 and MMP-9, MMP-13 is another ECM-degradative enzyme associated with HCC.[18] MMP-13 influences MMP cascades, both activating and being activated by several other MMPs such as MMP-2. Moreover, it has an exceptionally wide substrate specificity compared to other MMPs[24] and is capable of cleaving native fibrillar collagens of types I, II, III, V and IX, which are key molecules during liver fibrosis, a common phenomenon observed before and during the occurrence of HCC.

Saikosaponin D (SSd) is a major component of Chai Hu (Bupleurum Falcatum), which is used to treat various liver diseases in traditional Chinese medicine. This triterpene saponin reportedly induces pleiotropic biological effects, such as anti-inflammatory and anti-convulsive induction of differentiation, immuno-modulatory and antiproliferative activities. Our previous studies have shown that SSd significantly reduced hepatocarcinogenesis,[25] angiogenesis[26] and liver fibrosis[27] in animal models, indicating that SSd may inhibit the occurrence, development and metastasis of HCC through multi-cascade effects. However, the mechanism details are still largely unknown.

In this study, we investigated effects of SSd on the expression of syndecan-2 and MMP-2, MMP-13, and TIMP-2 in liver tissue from a rat HCC model established using N-diethylnitrosamine (DEN) treatment. We found that SSd can inhibit the expression of these molecules involving the occurrence, development and metastasis of HCC.

METHODS

All chemicals were obtained from Xi'an Chemical Reagent Factory (Xi'an, China) unless otherwise stated, and were of analytical grade or better.

Animal model preparation

The experiment was approved by the Animal Ethics Committee, Medical School of Xi'an Jiaotong University (Xi'an, China). Use of animals in this study was in accordance with the National Institute of Health publication 85-23 "Guide for Care and Use of Laboratory Animals" (National Research Council, 1996).[28] Fifty male Sprague Dawley (SD) rats weighing (248 ± 12) g (3-4 months old) were purchased from the Laboratory Animal Center of the Medical School, Xi'an Jiaotong University. Rats were acclimated for 7 days, then randomly divided into 3 groups: controls (n=10), model group (n=20) and SSd group (n=20). Rats in model and SSd groups received 0.2% (w/v) DEN (Sigma-Aldrich Co., St. Louis, MO, USA) in saline (10 ng of DEN/g body weight/day) intragastrically every 5 days for 16 weeks; 0.9% (w/v) normal saline was administered to control rats. Simultaneously, 0.03% (w/v) SSd in saline was administered daily to the SSd group by intraperitoneal injection at 1.5 mg/kg body weight. Distilled water was accessible freely to all rats.

Sample collection

The weights of the rats were measured every week. At 16 weeks after the initiation of experiment, all the rats were scarified under general anesthesia. Hepatic tissues

were collected and fixed in 4% (w/v) paraformaldehyde in phosphate buffered saline (0.16M NaCl, 0.003M KCl, 0.008M Na_2HPO4, 0.001M KH_2PO_4, pH 7.3, PBS). The tissues were embedded in paraffin and sectioned at 8-μm thickness.

Histological staining

After deparaffinization, sections were stained with hematoxylin and eosin (H&E) as usual. After dehydration sections were mounted using DPX mounting medium (Thermo Fisher Scientific, Loughborough, UK). Representative regions were photographed under bright field optics using a Leica DMRB light microscope (Leica, Wetzlar, Germany) equipped with digital image acquisition.

Immunohistochemical staining

Immunohistochemical staining was performed using the Mouse on Mouse™ (for syndecan-2, Vector labs, Peterborough, UK) or Vectastain®, Elite® ABC Kits (for MMP-2, MMP-13 & TIMP-2, Vector labs, Peterborough, UK) according to the manufacturers' protocols. Briefly, sections were incubated with 0.3% (v/v) hydrogen peroxide for 30 min at room temperature to quench endogenous peroxidase activity. After blocking with mouse immunoglobulin (Ig) blocking reagent for 1 h or 2.5% (v/v) normal horse serum for 30 min at room temperature, sections were incubated with rat anti-syndecan-2 (Novus Biological, Cambridge, UK), rabbit anti-MMP-2 (Abcam, Cambridge, UK), goat anti-MMP-13 (Santa Cruz Biotechnology, Ltd., Santa Cruz, CA, USA) or rabbit anti-TIMP-2 (Abcam, Cambridge, UK) primary antibody respectively for 60 min. For the negative control, the primary antibody was replaced by PBS, 2 μg/mL rabbit IgG (DAKO, Cambridge, UK) or 2 μg/mL goat IgG (DAKO, Cambridge, UK). Sections were then incubated with biotin labeled horse anti-rat, rabbit, or goat IgG for 30 min at room temperature. After washing, sections were incubated with Vectastain ABC reagent for 30 min. Sections were then visualised using Vector NovaRED™ kit (Vector labs, Peterborough, UK) according to the manufacturer's protocols. Cell nuclei were counterstained with hematoxylin. After dehydration, sections were mounted using DPX mountaining medium. Representative regions were then photographed under bright field optics using a Leica DMRB bright field microscope equipped with digital image acquisition.

Quantitative analysis for tissue staining rates

Positive staining in cells was quantitatively analyzed. Sections of 4 liver tissues from 4 individual rats in each experimental group were taken for analysis. For each tissue, three sections were randomly selected, and positively and negatively stained cells in these sections were counted using Image J software (National Institutes of Health, Bethesda, MD, USA). The percentage of positive cells was then calculated as: [(positive stained cells)/(positive stained cells+negative stained cells)]×100%.

Semi-quantitative analysis for the intensity of MMP-2 positive staining in the tissue

Tissue MMP-2 $^+$ staining was analyzed by Integrated Optical Density (IOD) using the Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Bethesda, MD, USA) as described previously[29] with minor modification. Briefly, four 40 × TIFF-format images from four individual rats in each group was analyzed in a blinded manner. All images were taken with the same microscope and camera setting. The Image-pro Plus software was used to calculate the average IOD per stained area (μm^2) (IOD/area) for MMP-2^+ staining.

Statistical analyses

Data are presented as mean ± S.E.M, with samples derived from 4 animals in each group. Data were tested for normality and equal variance. Student's *t* test or one-way analysis of variance (ANOVA) plus Bonferroni's post-test was carried out using GraphPad Prism 4.0 software (GraphPad Software Inc, La Jolla, CA, USA). $P \leq 0.05$ was considered significant.

RESULTS

SSd reduced the number of malignant nodules and hepatoma malignancy grade

Sixteen weeks after administering DEN, malignant nodules were observed on surfaces of livers from rats in model group, on average. The number of macroscopic modules larger than 3 mm and 5 mm observed on the surface of each liver tissues in the model group averaged 33.4 and 4.9 respectively (the largest was approximately 1.5 cm × 1.0 cm × 0.8 cm); whereas in the SSD group, the numbers of macroscopic nodules bigger than 3 mm and 5 mm on single liver from SSd group averaged significantly less—21.7 and 2.4, respectively (Table 1, $P \leq 0.01$). H&E staining was used to identify and classify the cancerous nodules pathologically according to Edmondson.[30] Hepatoma nodules in both model and SSd groups were evident (Figure 1B & C). However, the model group had more nodules than the SSd group, similar with the macroscopic observation described above. Hepatoma nodules in the model and SSd groups were isolated by fibroblasts, which are believed to produce different collagen fibers to form fibrosis. Interestingly, the fibrosis observed in the model group (Figure 1E) was evidently thicker than that in the SSd group (Figure 1F). HCC cells dedifferentiation was also investigated according to Edmondson.[30] All cancer cells in the model group (Figure 1H) were classified as grade III whereas the cells in SSd group were evaluated as grade I – II (Figure 1I). There were no macroscopic malignant nodules in the control-group livers, which showed normal morphology and liver tissue structure (Figure 1A, D & G). This re-

sult indicated that SSd inhibits DEN-induced carcinogenesis and cancer development in rats. To further address its mechanism, we investigated the expression of syndecan-2 in the liver tissues from these three groups.

Table 1 The number and size of malignant nodules in rat liver tissue

Group	*n*	Nodules ≥3mm	Nodules ≥5mm	The biggest nodule (mm^3)
Control group	10	0	0	0
Model group	14	33.4±7.9	4.9±1.9	122.8
SSd group	19	21.7±5.1[a]	2.4±1.3[a]	39.6

Notes: [a]*P*≤0.01 when compared with model group.

SSd significantly decreased syndecan-2 expression in HCC tissues

Syndecan-2 expression in liver tissue was investigated using immunochemical staining. There was no apparent syndecan-2$^+$ staining in the liver tissue from control group, either in portal area, central vein area or hepatic lobule (Figure 2A&D). However, apparent syndecan-2$^+$ liver tissue was seen in the model group, especially around in the cytoplam of hepatoma cells in the central vein (Figure 2B, black arrow—enlarged image is shown in the small insert). Moreover, yndecan-2$^+$ staining was also observed in the cytoplasm and/or cell membranes in hepatoma cells far from portal area and central vein (Figure 1-ii). Statistical analysis showed much higher syndecan-2$^+$ staining in the model group (35.2%) than in the control group (no staining at all, herein 0%) (*P*≤0.001). In SSd group, syndecan-2$^+$ staining was still mainly seen in hepatoma cytoplasm around portal area and central vein (Figure 2C). However, in hepatoma cells far from these areas, syndecan-2 staining was predominately seen on the cell membranes instead of cytoplasm (Figure 2F). Syndecan-2$^+$ staining in SSd group was approximately 16.5%, significantly lower than in the model group (*P*≤0.001), but higher than in the control group (*P*≤0.001). These results indicated that DEN-induced HCC in rat liver increases syndecan-2 expression in cells, whereas SSd treatment reduces syndecan-2 expression, suggesting a correlation between HCC and syndecan-2 expression. To further address the mechanism, we investigated enzymes that affect HCC development and metastasis, such as MMP-2, MMP-13 and TIMP-2.

SSd significantly decreased MMP-2 expression in HCC tissues

In controls, MMP-2$^+$ staining was evenly distributed in hepatocyte cytoplasm (Figure 3A&D), but was more intensive in hepatoma cells, not only in cytoplasm but also in some cell nuclei (Figure 3B&E). Interestingly, MMP-2 staining in fibroblasts that form fibrosis around hepatoma nodules and central vein was apparently weaker than in hepatoma cells. In SSd-group tissue, MMP-2$^+$ staining was seen in some but not all hepatoma cytoplasm (Figure 3-III and iii). Unlike the model group, MMP-2$^+$ staining was also seen in fibroblasts forming fibrosis around hepatoma nodules (Figure 3C). Semi-quantitative analysis indicated higher MMP-2$^+$ staining intensity in model group than that in controls (0.37 vs 0.27, *P*≤0.01). The SSd group showed significantly reduced MMP-2 staining compared to the model group (0.31 vs 0.37, *P*≤0.05), but higher MMP-2 staining than in the control group (0.31 vs 0.27, *P*≤0.05).

SSd significantly decreased MMP-13 expression in HCC tissues

MMP-13 expression, which was also investigated using immunohistochemical staining, was mainly distributed on cell membranes in some but not all control-group hepatocytes (Figure 4D). Noticeably, intensive MMP-13 staining was observed in cytoplasm of 1-2 layers of cells around the central vein and portal area (Figure 4A). In the model group, MMP-13$^+$ staining was observed in most of the hepatoma cytoplasm and cell membranes, especially in cells around central vein and portal area (Figure 4B&E). Statistical analysis showed the model-group MMP-13$^+$ staining rate (72.55%) to be higher than in the control group (12.55%, *P*≤0.001). SSd-group MMP-13 staining was mainly seen on cell membranes instead of cytoplasm in hepatoma cells distant from central vein and portal area (Figure 4E), similar to that observed in controls. SSd-group MMP-13$^+$ cells around central vein and portal areas were dramatically fewer than in the model group, almost similar to the control group (Figure 4-III). The overall MMP-13$^+$ rate in SSd group (20.18%) was less than in the model group (72.55%, *P*≤0.01), but higher than for controls (12.55%, *P*≤0.05).

SSd significantly decreased TIMP-2 expression in HCC tissues

In control samples, TIMP-2$^+$ staining was seen in cells around portal and central vein areas (Figure 5A), and was also evident on cell membranes and in cytoplasm in some cells in the hepatic plate (Figure 5D). The overall TIMP-2$^+$ rate in control-group hepatic cells from was approximately 20.9%. In the DEN-induced HCC model group, major TIMP-2$^+$ staining was seen in fibroblasts forming fibrosis around HCC nodules, and cells in the portal and central vein areas (Figure 5B). TIMP-2$^+$ staining was also seen in cytoplasm and on cell membranes of some hepatoma cells (Figure 5E), and was much higher for model-group hepatoma cells, at 57.2%, than t in the control group (*P*≤0.001). TIMP-2 staining in the SSd group was similar to that in the model group, mainly observed in the cells around portal and central vein areas (Figure 5B),and in fibroblasts forming fibrosis in liver tissue (Figure 5F),

although it was much thinner than in model the group. TIMP-2$^+$ staining in some hepatoma cells was similar to those in the control group, mainly localized on cell membranes and cytoplasm (Figure 5F). The SSd-group TIMP-2$^+$ rate was significantly less than that in the model group (P≤0.01). However, no difference was observed between control and SSd groups (P>0.05).

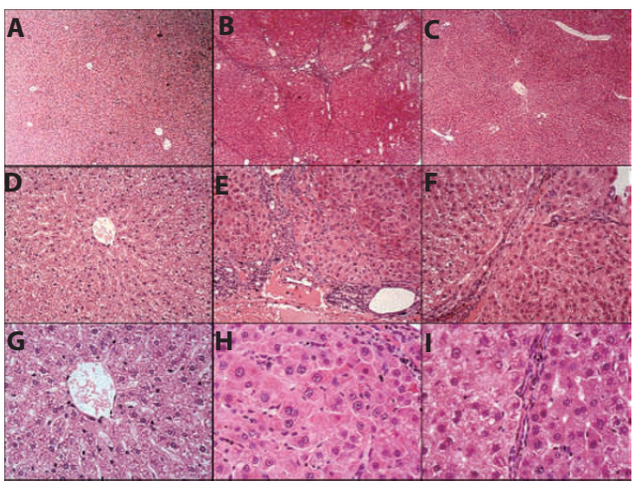

Figure 1 H&E staining results for liver tissue from control (A, D, G), model (B, E, H) and SSd groups (C, F, I)

Normal liver structure and cell morphology were observed in the control group. However, apparent hepatoma nodules and fibrosis in model group were evident when compared with the control group. SSd reduced hepatoma nodule formation and fibrosis compared with the model group. Hepatoma cells in model group were graded as III whereas those in the SSd group were grade I-II according to Edmondson. Scale bars: 400 μm (A – C); 100 μm (D – F); 50 μm (G – I).

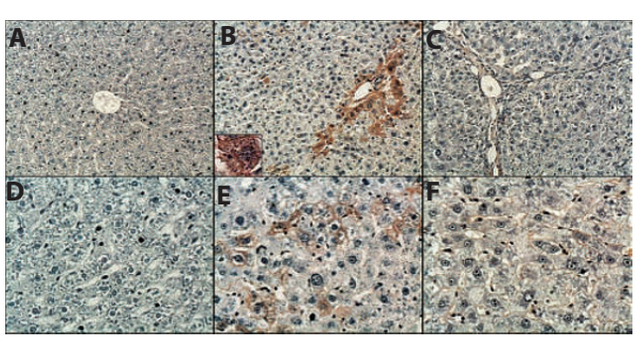

Figure 2 Syndecan-2 immunohistochemical staining results in rat liver tissues

Rats were treated with DEN with or without SSd for 16 weeks to establish a hepatocellular carcinoma animal model. Liver tissues were harvested and sectioned. Red: Syndecan-2 expression. Blue: cell nuclei, counterstained by hematoxylin. A&D: control group; B&E: model group; C&F: SSd group. Scale bars: 100 μm (A-C); 50 μm (A, E &F).

DISCUSSION

Several rat models for liver carcinogenesis have been described previously.[31] However, unlike other models, the DEN-induced HCC model reproduces the sequence of cirrhosis and HCC, occurring as in human HCC development.[32] In this study, we used the DEN-induced rat HCC model to mimic human HCC pathogenesis.

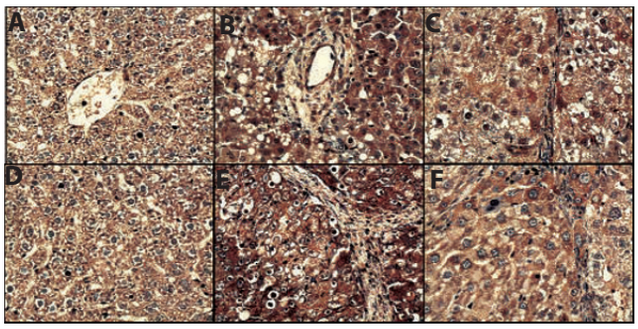

Figure 3 MMP-2 immunohistochemical staining results in rat liver tissues

Rats were treated with DEN with or without SSd for 16 weeks to establish a hepatocellular carcinoma animal model. Liver tissues were harvested and sectioned. MMP-2 expression was stained by red color. Cell nucleuses were counterstained by hematoxylin (blue color). A&D: control group; B&E: model group; C&F: SSd group. Scale bar: 50 μm.

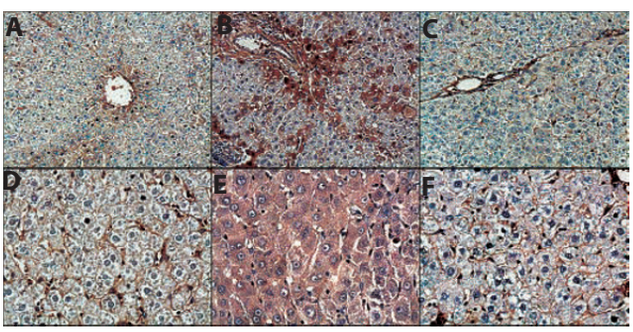

Figure 4 MMP-13 immunohistochemical staining in rat liver tissues

Rats were treated with DEN with or without SSd for 16 weeks to establish a hepatocellular carcinoma animal model. Liver tissues were harvested and sectioned. Red: MMP-13 expression. Blue: cell nuclei, counterstained by hematoxylin. A&D: control group; B&E: model group; C&F: SSd group. Scale bar: 100 μm (A-C); 50 μm (A,E&F).

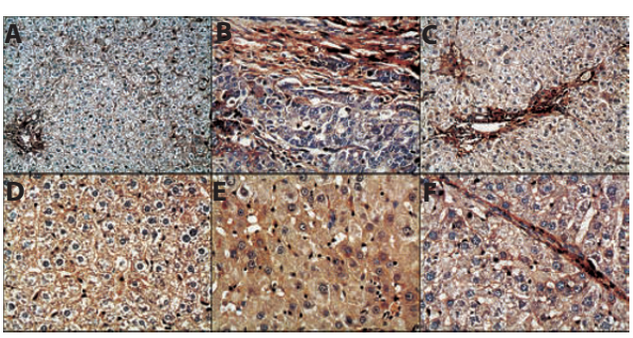

Figure 5 TIMP-2 immunohistochemical staining results in rat liver tissues

Rats were treated with DEN with or without SSd for 16 weeks to establish a hepatocellular carcinoma animal model. Liver tissues were harvested and sectioned. Red: TIMP-2 expression. Blue: cell nuclei, counterstained by hematoxylin. A&D: control group; B&E: model group; C&F: SSd group. Scale bars: 100 μm (A-C); 50 μm (A, E&F).

SSd, an herb used in traditional Chinese medicine to treat liver disease, was administered to rats to address its potential mechanism. The general condition of control rats was "normal" as expected. However, the DEN-treated rats had apparent weight reduction, lags in response, and decreased activity and diet intake, es-

pecially in the model group. By 16 weeks into this study, six model-group rats had died (30%) but only one died in the SSd group (5%), indicating that SSd could reduce mortality in rats with HCC. Moreover, histological analysis indicated that SSd also decreased hepatoma nodules and HCC malignancy. Although these results suggest that SSd partially inhibits DEN-induced HCC in a rat model, the mechanism is not clear.

Syndecan-2 is a multimeric cell-surface protein with complex functions. The relationship between syndecan-2 and carcinogenesis has been implied in in lung, prostate, and colon cancers[33,34] in which syndecan-2 expression was found to be elevated. However, syndecan-2 expression in HCC has been little studied. In the present study, we found no syndecan-2 expression in control-group liver tissues. Surprisingly, intensive syndecan-2 staining was seen in liver tissue of rats with DEN-induced HCC, especially in cells around the central vein and portal areas, suggesting that syndecan-2 affects HCC occurrence, development and metastasis. Although the precise mechanism is unclear, regulation of tumor cell adhesion and migration, and facilitation of angiogenesis may be involved. Reportedly, syndecan-2 is required for lung carcinoma cell adhesion to fibronectin; reduced syndecan-2 expression in those cells decreases focal adhesion formation and migration.[35] Moreover, overexpressed syndecan-2 in colon carcinomas increases cell proliferation, piling-up of cells in culture[36,37] and invasive phenotypes.[11] All these results suggest that syndecan-2 overexpression on cell surfaces enhances carcinoma growth and migration through interaction with ECM components such as fibronectin—a molecule also upregulated in HCC, especially on endothelial blood vessel surfaces in cancerous tissue.[38] Our immunohistochemical results are consistent with this premise, with no syndecan-2 expression in normal liver tissue but significantly higher syndecan-2 staining in DEN-induced HCC tissue, especially in the cells around central vein and portal area, where are areas of the liver that are both highly vulnerable to HCC metastasis[39] and that highly express fibronectin.[40] Interestingly, SSd significantly decreased DEN-induced syndecan-2 expression in liver tissue, suggesting that SSd inhibits HCC by downregulating syndecan-2. Our earlier experiments showed that SSd could inhibit angiogenesis in different animal models by down-regulating expression of VEGF,[26,27] a ligand that can bind to syndecan-2 and thusinduce angiogenesis. Syndecan-2 downregulation reduces spreading and adhesion of endothelial cells, and impairs formation of capillary-like structures, suggesting its necessity in angiogenesis.[41] Therefore, SSd may inhibit HCC angiogenesis by reducing the expression of syndecan-2 and its VEGF binding. Further studies are needed to address this mechanism.

MMPs are a family of extracellular endopeptidases that selectively degrade ECM components and are thus thought to contribute to tumor cell invasion. Several studies showed that different MMPs, including MMP-2, 7, 9, and 13, play important roles in HCC.[19,21,42-44] In this study, we found that the expression of MMP-2 and MMP-13 in DEN-induced HCC liver tissue were much higher than in normal liver tissue, suggesting that these two enzymes affect HCC occurrence and metastasis, which is consistent with prior studies.[18] Interestingly, SSd treatment significantly reduced expression of these enzymes in liver tissue, which correlates with alleviation of HCC malignancy; this suggests that SSd may prevent HCC invasion and metastasis by inhibiting activities of MMP-2 and MMP-13. MMP-2 is a gelatinase, which degrades type IV collagen, the major structural component of basement membranes. Unsurprisingly, HCC metastasis is apparently related to MMP-2 expression, as basement membrane destruction is a key step in angiogenesis.[45] MMP-13 is another collagenase, widely expressed in many pathological conditions. It is apparently central to the MMP cascade—both activating and being activated by other MMPs (MMP-14, -2 and -3)— and to collagen remodeling in the angiogenic cascade.[46] SSd may thus alleviate HCC development and metastasis by inhibiting MMP-2 and MMP-13 in tumoral angiogenesis.

MMP activity is specifically inhibited by the so-called tissue inhibitors of metalloproteases (TIMPs). Currently, four different TIMPs are known to exist: TIMPs-1, -2, -3 and -4. TIMPs mediate ECM homeostasis by regulating MMP activity, and may play important roles in chronic liver disease.[47] If TIMPs can inhibit MMPs in vivo, their expression might be expected to be downregulated in HCC. However, TIMP-1 and/or 2 overexpression reportedly leads to increased hepatoma cell migration, and is associated with invasion and metastases in HCC,[48] and shortened overall survival of HCC patients.[18] Therefore, TIMP expression in HCC is a likely marker for dynamic ECM remodeling, which is dependent on the ratio between MMPs and TIMPs. This premise is consistent with the correlation between the MMP-2/TIMP-2 imbalance and metastasis and prognosis in patients with HCC. Moreover, cirrhosis generally occurs in HCC,[32] which is usually accompanied with a high expression of TIMP-2.[49] In this study, the most intensive TIMP-2 staining was seen in fibroblasts instead of hepatoma cells, which differs from the control group, and further illustrates the role of TIMP-2 in HCC-associated cirrhosis. SSd reduced TIMP-2 expression in liver tissue compared with that in the model group, suggesting that SSd treatment slows ECM remodeling and alleviates cirrhosis. This result also supports the view that MMPs and TIMPs do more than degrade or build the ECM, and are multifunctional proteins affecting cell behavior, cell – cell communication and tumor angiogenesis.[50]

In summary, our data show elevated syndecan-2 expression in HCC tissue; SSd — which is used to treat chronic liver disease in traditional Chinese medicine — apparently counters HCC by reducing expression of syndecan-2, MMP-2, MMP-13 and TIMP-2 in liver

tissue. Although the mechanism is unclear, SSd may inhibit carcinogenesis by reducing cell-ECM interaction, thus slowing ECM remodeling and angiogenesis in HCC. Whether syndecan-2 expression affects that of MMP and TIMP-2 in HCC should be further investigated, and may provide future therapeutic targets for this common tumor.

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