GalNAc-T14 may be involved in regulating the apoptotic action of IGFBP-3

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Insulin-like growth factor binding protein-3 (IGFBP-3) is known to induce apoptosis in an insulin-like growth factor (IGF)-dependent and IGF-independent manner, but the mechanism underlying the IGF-independent effects remains unclear. Polypeptide *N*-acetylgalactosaminyltransferase 14 (GalNAc-T14) is a novel IGFBP-3 binding partner. In this paper, small interference RNA (siRNA) targeting GalNAc-T14 was used to examine whether GalNAc-T14 affects the apoptotic action of IGFBP-3. Using semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis, we determined that GalNAc-T14 expression was downregulated by the siRNA directed against GalNAc-T14. Apoptosis analysis of IGFBP-3-overexpressing cells treated with siRNA against GalNAc-T14 was performed to determine if GalNAc-T14 was specifically involved in IGFBP-3 signalling. The results, as determined by flow cytometric analysis and caspase-3 assay, showed that the extent of apoptosis induced by IGFBP-3 increased with RNA interference (RNAi) knockdown of GalNAc-T14. Our data suggest that GalNAc-T14 influences the apoptotic action of IGFBP-3 and might mediate the signalling pathway of IGFBP-3. Experiments to determine the role of GalNAc-T14 in the regulation of apoptosis induced by IGFBP-3 are under way.

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1. Introduction

Polypeptide *N*-acetylgalactosaminyltransferase 14 (GalNAc-T14, EC 2.4.1.41) belongs to a large subfamily of glycosyltransferases residing in the Golgi apparatus. *N*-acetylgalactosaminyltransferases (GalNAc-Tases) catalyse the first step in the *O*-glycosylation of mammalian proteins by transferring *N*-acetyl-D-galactosamine (GalNAc) to peptide substrates (Brockhausen 1999). GalNAc-Tase activity was first reported 39 years ago by McGuire and Roseman (1967). To date, 15 GalNAc-Tases (GalNAc-T1–T15) have been identified in mammals. GalNAc-T14 was

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Abbreviations used: AMF/PGI, autocrine motility factor/phosphoglucose isomerase; DMEM, Dulbecco modified Eagle medium; ECL, enhanced chemiluminescence; FCS, foetal calf serum; GalNAc, *N*-acetyl-D-galactosamine; GalNAc-T14, EC 2.4.1.41, polypeptide *N*-acetylgalactosaminyltransferase 14; GalNAc-Tases, *N*-acetyl-galactosaminyltransferases; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HRP, horseradish peroxidase; Ig, immunoglobulin; IGF, insulin-like growth factor; IGFBP-3, insulin-like growth factor binding protein-3; NC, negative control; OD, optical density; PBS, phosphate buffered saline; PI, propidium iodide; PS, phosphatidylserine; RCC, renal cell carcinoma; RNAi, RNA interference; RT-PCR, reverse-transcriptase polymerase chain reaction; RXRα, retinoid X receptor-α; siRNA, small interference RNA

first reported in 2003 by Wang *et al.* (2003), who cloned its cDNA and designated it as GalNAc-T14, and confirmed that it is a new member of the GalNAc-Tase family. Quantitative real-time polymerase chain reaction (PCR) analysis revealed that the GalNAc-T14 transcript was highly expressed in kidney tissue, which suggests that GalNAc-T14 might be involved in *O*-glycosylation in the kidney (Wang *et al.* 2003). *O*-glycosylation is an important post-translational modification, and the GalNAc-Tase family is closely related to invasion, metastasis and proliferation of many cancer cells (Brockhausen 1999).

Insulin-like growth factor binding protein-3 (IGFBP-3) is known to inhibit cell proliferation and induce apoptosis through insulin-like growth factor (IGF)-dependent and IGF-independent mechanisms, but the mechanism underlying the IGF-independent effects is not yet clear (Rajah *et al.* 1997; Sawada *et al.* 1994). To understand the molecular mechanism by which IGFBP-3 induces apoptosis in an IGF-independent manner, it is essential to study the cellular proteins interacting with IGFBP-3. In an earlier work, we utilized the yeast two-hybrid assay to screen a novel IGFBP-3 binding partner from a human foetal liver cDNA library. The interaction between GalNAc-T14 and IGFBP-3 was identified *in vitro* and *in vivo*. IGFBP-3 itself possesses proapoptotic and antiproliferative activity in a variety of cancer cell lines, and GalNAc-T14 is a new member of the GalNAc-Tase family, which is closely related to invasion, metastasis and proliferation of many carcinomatous cells (Brockhausen 1999). This information implies that interaction between IGFBP-3 and GalNAc-T14 may play a significant role in the modulation of carcinomatous cell proliferation and apoptosis. We investigated the association between IGFBP-3 and GalNAc-T14 to determine if it is functionally significant. We found that GalNAc-T14 may be involved in the regulation of apoptosis induced by IGFBP-3 in 786-O cells. Using GalNAc-T14 small interference RNA (siRNA), we demonstrate that GalNAc-T14 has an important role in IGFBP-3-induced apoptosis.

2. Materials and methods

2.1 *Cell lines and antibodies*

Human renal cell carcinoma (RCC) 786-O cells were grown in Dulbecco modified Eagle medium (DMEM; Gibco) supplemented with 10% (v/v) foetal calf serum (FCS). The following antibodies were used: polyclonal antibody against human polypeptide GalNAc-T14 (prepared by our laboratory) and glyceraldehyde 3-phosphate dehydrogenase (GADPH; Santa Cruz, CA, USA). GalNAc-T14 Stealth™ Select RNA interference (RNAi) (set of 3 oligos) was purchased from Invitrogen (CA, USA). Transfection of siRNA was done using Lipofectamine 2000 (Invitrogen, CA, USA) according to the manufacturer's instructions.

2.2 *Cell culture and siRNA transfection*

786-O cell lines were seeded at 3×10^5/well in 6-well plates 1 day before transfection. After 24 h, cells were transfected using Lipofectamine 2000 reagent and 25 nM of siRNA targeting GalNAc-T14. The three GalNAc-T14 target sequences used were: siRNA1, 5′-UUAUUGCGCAAGCA UUUCACCUUGG-3′ (sense) and 5′-CCAAGGUGAAAUG CUUGCGCAAUAA-3′ (antisense); siRNA2, 5′-UAUGAU AGGAGUCCUGAUGGGCUCC-3′ (sense) and 5′-GGAGC CCAUCAGGACUCCUAUCAUA-3′ (antisense); siRNA3, 5′-UGUCCAUGUCCAUAUCAUAUUUCCC -3′ (sense) and 5′-GGGAAAUAUGAUAUG-GACAUGGACA-3′ (antisense). Negative control (NC) siRNA was included. Forty-eight hours after transfection, the efficiency of the knockdown was analysed at the mRNA level using semi-quantitative reverse-transcriptase (RT)-PCR. RNA interference (RNAi) experiments were repeated thrice.

2.3 *Total RNA preparation and semi-quantitative RT-PCR assay*

1×10^6 786-O cells transfected by three siRNAs targeting GalNAc-T14 or NC were harvested and total RNA was extracted with Trizol reagent (Invitrogen, CA) according to the manufacturer's instructions. RT-PCR was performed with 0.5 μg of total RNA using an RNA PCR™ kit (AMV) version 3.0 (TaKaRa, Japan) according to the manufacturer's instructions: 1 cycle at 42°C for 60 min, 99°C for 5 min, and 5°C for 5 min for reverse transcription; 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min for 25 cycles for PCR. The oligonucleotide primers used in the PCR amplifications are as follows: GalNAc-T14, 5′-TAGCATCATCATCACCTTCCAC-3′ (sense) and 5′-TTACAGTCATCAGGGTCATTGC-3′ (antisense); GAPDH, 5′-GGTCGGAGTCAACGGATTTG-3′ (sense) and 5′-ATGAGCCCCAGCCTTCTCCAT-3′ (antisense). After the PCR amplification, 9 μl of the samples with 1 μl of a tracking dye were run on a 1.5% agarose gel containing 1 μg/ml ethidium bromide. The PCR products were scanned (Uvidoc, Gel Documentation System, Cambridge, UK) and the amount of PCR products present in each lane was determined using the Molecular Analyst software (Bio-Rad, Philadelphia, PA, USA) version 1.4.

2.4 *Western blot analysis*

Seventy-two hours after treatment with NC siRNA or siRNA1, cells were harvested according to the above method. Cytoproteins were separated by SDS–PAGE in

12% (w/v) polyacrylamide gels. Western blot was performed using anti-GalNAc-T14 antibody as the primary antibody, followed by anti-rabbit immunoglobulin (Ig)G conjugated with horseradish peroxidase (HRP) (BioLab), and visualized by an enhanced chemiluminescence (ECL) kit (Amersham Biosciences).

2.5 *GalNAc-T14 affected the apoptosis induced by IGFBP-3*

786-O cells were co-transfected with GalNAc-T14 siRNA and IGFBP-3 to determine the effect of GalNAc-T14 knockdown on IGFBP-3-induced apoptosis. 786-O cells were cultured in DMEM (Gibco, Grand Island, New York) supplemented with 10% FCS (HyClone, Hampton, NH), 2 mM L-glutamine, 20 U/ml penicillin, and 20 mg/ml streptomycin. The cultures were kept in an incubator with 5% CO_2 and 95% humidified air at 37°C. Cells were seeded in 6-well culture plates, and then grown to 50–70% confluence. pCMV2-Myc (Clontech) with or without a full-length human IGFBP-3 cDNA was transfected transiently into 786-O cells, respectively, using Lipofectamine2000 according to the manufacturer's instructions. The expression of a 42 kDa IGFBP-3 protein was determined 48 h after transfection by western blot. At the same time, caspase-3 activity assay was used to detect the apoptosis induced by IGFBP-3.

Subsequently, 786-O cells were transfected transiently with siRNA1 against GalNAc-T14 or NC siRNA using Lipofectamine 2000. After 24 h, pCMV-IGFBP-3 and siRNA1 were co-transfected into the 768-O cells. After incubation for 6 h, each dish was replaced with 2 ml complete medium and incubated for 48 h, while the cells were transfected with siRNA1 or NC siRNA or both pCMV-IGFBP-3 and NC siRNA as controls. At the same time, a western blot was performed to detect the expression of IGFBP-3.

2.7 *Detection of apoptosis*

Apoptotic cells were quantified by measuring externalized phosphatidylserine (PS) assessed by uptake of annexin V-EGFP and propidium iodide (PI). After various experimental treatments, cells were stained with an annexin V-EGFP apoptosis detection kit (Nanjing Keygen Biotech. Co., Ltd.). Briefly, harvested cells were rinsed once with phosphate buffered saline (PBS), and then resuspended in 250 μl of 1× binding buffer and 2 μl annexin V-EGFP, 2 μl of PI, and then incubated at room temperature for 15 min in the dark. For flow cytometry analysis, cells were stained with annexin/PI, and the population of apoptotic cells was analysed immediately by flow cytometry (FACSCalibur, BD Bioscience).

2.8 *Caspase-3 activity assay*

Activation of the caspase-3 proteolytic cascade was measured by a caspase-3 colorimetric assay kit (Nanjing Keygen Biotech. Co., Ltd.) according to the manufacturer's protocol. Briefly, after treatment, cells (3–5) $\times 10^5$ were harvested and washed twice with PBS and resuspended in 50 μl of ice-cold lysis buffer. After 20 min of incubation on ice, cell lysates were clarified by centrifugation (10 000 *g* for 1 min at 4ºC), and Bradford assay was performed on the supernatant to determine the protein concentration. Fifty millilitres of the supernatant containing 150 μg of total protein was mixed with 2× reaction buffer and 5 μl of caspase-3 substrate, and then incubated at 37ºC for 4 h in the dark. The optical density (OD) values at 405 nm were read by a Model 550 Microplate Reader (Bio-Rad). The activation of caspase-3 was calculated by OD (test)/OD (control).

3. Results

3.1 *GalNAc-T14 gene expression analysis*

To assay the efficiency of GalNAc-T14 siRNA interference, three siRNAs were transfected into 786-O cells. As expected, knockdown of GalNA-T14 decreased mRNA expression as measured by semi-quantitative RT-PCR. Figure 1A shows that siRNA1 and siRNA3 had higher interference efficiency than siRNA2; therefore, siRNA1 was chosen for subsequent experiments. Semi-quantitative RT-PCR with GAPDH served as a control (figure 1B).

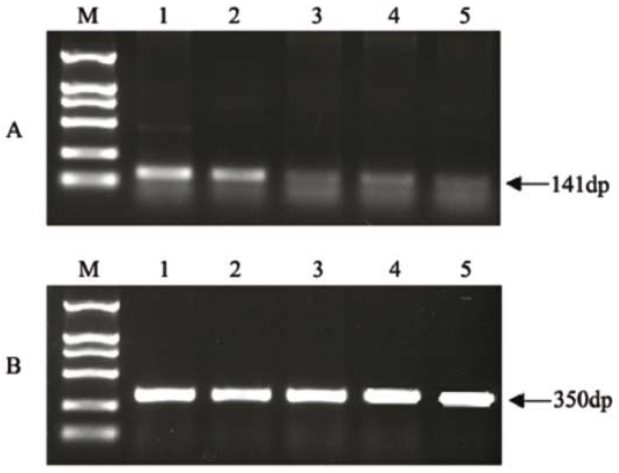

Figure 1. The expression of GalNAc-T14 mRNA of 786-O cells transfected with different small interference RNAs (siRNAs) against GalNAc-T14 or negative control siRNA by semi-quantitative RT-PCR. **(A)** Semi-quantitative RT-PCR of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as a control. **(B)** M, DNA marker (DL2000); 1, normal 786-O cells; 2, negative control siRNA; 3, siRNA1; 4, siRNA2; 5, siRNA3.

Knockdown of GalNAc-T14 was also analysed at the protein level by western blot (figure 2). Compared with the control, knockdown of GalNAc-T14 decreased protein expression.

3.2 *Effect of IGFBP-3 on caspase-3 activity*

Caspase-3 has been identified as a key mediator of apoptosis in mammalian cells (Kothakota *et al.* 1997). To study the effect of IGFBP-3 alone on apoptosis, 786-O cells were transfected with IGFBP-3. Forty-eight hours after transfection, cells were collected and caspase-3 activity was measured by spectrophotometry. The expression of IGFBP-3 protein of 786-O cells transfected by pCMV-IGFBP-3 was determined by western blotting (figure 3A). The activity of caspase-3 in 786-O cells increased by more than 200% compared with the control (figure 3B).

3.3 *Inactivation of GalNAc-T14 increased the apoptosis induced by IGFBP-3*

To investigate whether GalNAc-T14 was implicated in IGFBP-3-induced apoptosis, we compared the apoptosis induced by IGFBP-3 with or without RNAi of GalNAc-T14. 786-O cells were transfected by GalNAc-T14 siRNA1 or NC siRNA. After 24 h, the transfected cells were transfected with IGFBP-3. The result of western blot showed that the overexpression of IGFBP-3 in 786-O cells remained the same (figure 4A). Cells were harvested and then stained with annexin V/PI and the apoptotic cell population was analysed immediately by flow cytometry. As shown in figure 4B, no significant changes in the apoptotic cell population

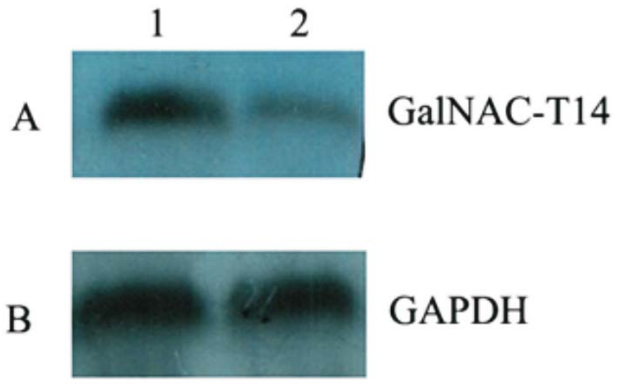

Figure 2. The expression of GalNAc-T14 protein of 786-O cells transfected by small interference RNA (siRNA1) against GalNAc-T14 or negative control siRNA determined by western blot. 1, Negative control siRNA; 2, siRNA1

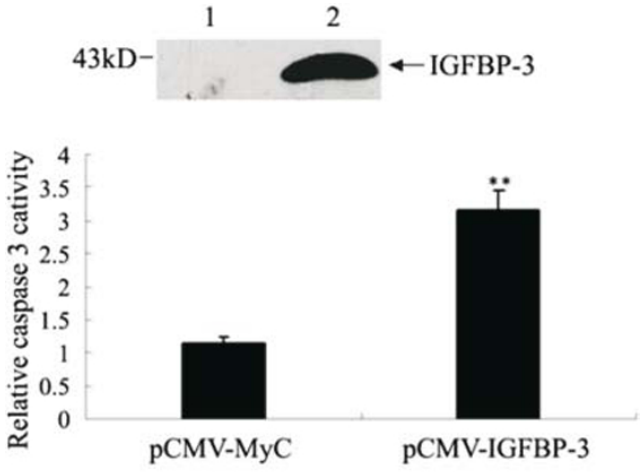

Figure 3. (A) Expression of the insulin-like growth factor binding protein-3 (IGFBP-3) protein of 786-O cells transfected by pCMV-IGFBP-3 or negative control plasmid determined by western blot. 1. Lysis of 786-O cells transfected by pCMV control; 2. Lysis of 786-O cells transfected by pCMV-IGFBP-3. **(B)** Caspase-3 activity after transfection with pCMV-IGFBP-3 or pCMV control, respectively. The OD_{405} values were measured and the caspase-3 activity was expressed relative to the untransfected cells, designated as 1. (***P*<0.01 compared with the pCMV-Myc control). The data presented were averaged from three independent experiments.

were observed for siRNA1 (22.67%) compared with NC siRNA (25.35%). This showed that GalNAc-T14 alone had no effect on apoptosis. In contrast, apoptosis induced by IGFBP-3 increased remarkably in GalNAc-T14 knockdown cells (52.39%) compared with the IGFBP-3 and NC siRNA co-transfected group (40.98%). These data indicate that inactivation of GalNAc-T14 increases the apoptosis induced by IGFBP-3. They also imply that GalNAc-T14 affects the apoptosis induced by IGFBP-3.

To further investigate whether the inactivation of GalNAc-T14 affected apoptosis induced by IGFBP-3, caspase-3 activity was measured in 786-O cells in which siRNA-mediated knockdown of GalNAc-T14 was performed while IGFBP-3 was overexpressed. The IGFBP-3 and NC siRNA co-transfected group, NC siRNA-transfected group and siRNA1-transfected group were included. As shown in figure 4C, the results generally agreed with those obtained by flow cytometry. The activity of caspase-3 in siRNA1-transfected cells was similar to that in NC siRNA-transfected cells. However, the activity of caspase-3 in 786-O cells co-transfected with IGFBP-3 and siRNA1 was about twice that compared with the IGFBP-3 and NC siRNA co-transfected groups (4.6 and 2.9, respectively). These results indicate that GalNAc-T14 has an important role in IGFBP-3-induced apoptosis.

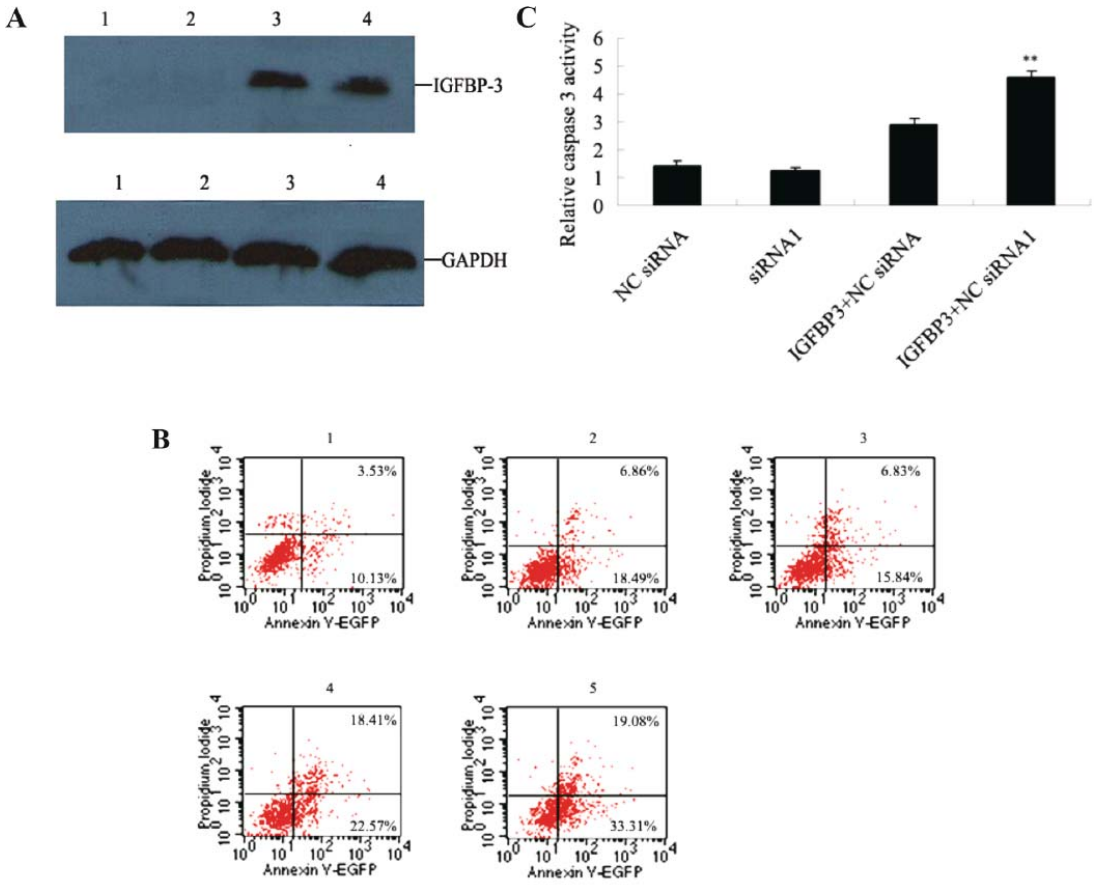

Figure 4. **(A)** Overexpression of insulin-like growth factor binding protein-3 (IGFBP-3) protein in 786-O cells transfected by normal control small interference RNA (siRNA) and/or pCMV-IGFBP-3, and siRNA1 and/or pCMV-IGFBP-3 determined by western blot (glyceraldehyde 3-phosphate dehydrogenase [GAPDH] as an internal control). 1. Lysis of 786-O cells transfected with negative control siRNA; 2. Lysis of 786-O cells transfected with siRNA1 against GalNAc-T14; 3. Lysis of 786-O cells transfected with pCMV-IGFBP-3 and negative control siRNA; 4. Lysis of 786-O cells transfected with pCMV-IGFBP-3 and siRNA1 against GalNAc-T14. **(B)** Seventy-two hours after transfection, cells were stained with annexin V-EGFP and propidium iodide (PI), and the apoptotic cells were analysed immediately by flow cytometry in a FACSCalibur. 1. Normal cells, 13.66% (3.53+10.13%) 2. NCdsRNA, 25.35% (6.86+18.49%) 3. siRNA1 22.67% (6.83+15.84%) 4. IGFBP-3 NCdsRNA 40.98% (18.41+22.57%) 5. IGFBP-3 siRNA1 52.39% (19.08+33.31%). **(C)** Caspase-3 activity. The OD_{405} values were measured and caspase activity was expressed relative to the untransfected control, designated 1. The data represent the mean of three independent experiments. Significant differences are indicated by asterisks. (**P<0.01 compared with IGFBP-3 and NC siRNA co-transfected control cells).

4. Discussion

Over the past decade, multiple lines of investigation have validated that IGFBP-3 is an inducer of cellular apoptosis, and that the effects that may be unrelated to its IGF binding (Firth and Baxter 2002). Most importantly, several groups have now reported successful *in vivo* treatment of cancer models with IGFBP-3, either as a single agent or in combination with chemotherapeutic agents (Kirman *et al.* 2004; Lee *et al.* 2004). Despite promising preclinical evidence of the use of IGFBP-3 as cancer therapy, questions remain as to the complex role of IGFBP-3 in various tumours. To better understand the molecular mechanism by which IGFBP-3 induces apoptosis in an IGF-independent manner, it is essential to study cellular proteins interacting with IGFBP-3. Several novel IGFBP-3 binding partners that may participate in its IGF-independent proapoptotic effects have been recently identified, such as fibronectin, autocrine motility factor/phosphoglucose isomerase (AMF/PGI), latent transforming growth factor β-binding protein-1 as well as the retinoid X receptor-α (RXRα) (Yamanaka *et al.* 1999; Liu *et al.* 2000; Gui and Murphy 2001; Mishra *et al.* 2004; Schedlich *et al.* 2004;). Recent reports indicate that candidate IGFBP-3 receptors (Delbe *et al.* 1991; Oh *et al.* 1993; Leal *et al.* 1997; Huang *et al.* 2003) and signal transduction pathways (Fanayan *et al.* 2000; Spagnoli

et al. 2002; Ricort 2004) have been described, but their physiological significance has not been established. Liu reported that RXRα was required for the apoptotic effects of IGFBP-3 in an F9 embryonic carcinoma cell line (Liu *et al.* 2000). However, a recent report described that IGFBP-3 can induce apoptosis in human prostate cancer cells without binding RXRα (Zappala *et al.* 2008). The molecular mechanisms responsible for the IGF-independent apoptotic actions of IGFBP-3 are not well understood. Clearly, more work needs to be done to clarify the IGF-independent mechanisms by which IGFBP-3 acts directly to induce apoptosis.

Earlier studies from our laboratory have shown that GalNAc-T14 is a novel binding partner for IGFBP-3 (Wu *et al.* 2007b) and the discovery of the binding of IGFBP-3 to GalNAc-T14 suggested that its apoptotic effects might be related to the interaction with GalNAc-T14. In order to ascertain whether the association between them is functionally significant, we performed RNAi knockdown of the GalNAc-T14 gene in 786-O cells, which are endogenously high expressers of GalNAc-T14 (Wu *et al.* 2007a). We selected three siRNA sequences from the region of GalNAc-T14 for evaluation. Out of them, siRNA1 showed better interference efficiency than the others by semi-quantitative RT-PCR and was chosen in subsequent experiments. Western blotting showed that protein expression of GalNAc-T14 was downregulated by GalNAc-T14 siRNA. We then performed apoptosis analysis of IGFBP-3-overexpressing cells treated with RNAi against GalNAc-T14. The results of caspase-3 assay showed that the activity of caspase-3 markedly increased in cells transfected with IGFBP-3, in contrast to cells transfected with NC. The results, as determined by flow cytometric analysis of annexin V/PI staining and caspase-3 assay, showed that the apoptosis of cells transfected with GalNAc-T14 siRNA alone was similar to that of cells transfected with NC siRNA. In other words, GalNAc-T14 itself could not induce apoptosis. The extent of apoptosis induced by IGFBP-3 increased in cells co-transfected with GalNAc-T14 siRNA and IGFBP-3, in contrast to cells co-transfected with NC siRNA and IGFBP-3, which showed that GalNAc-T14 might inhibit the apoptotic action of IGFBP-3 by interacting with it. Our observations suggest that GalNAc-T14 has an apparent effect on the apoptosis induced by IGFBP-3 in 786-O cells and may mediate the signal pathway of IGFBP-3.

We hypothesize that GalNAc-T14 mediates the apoptotic action of IGFBP-3 and regulates its activity by binding to it. One the other hand, it is likely that IGFBP-3 is O-glycosylated by GalNAc-T14, which changes the bioactivity of IGFBP-3. IGFBP-3 is known to be secreted as a glycoprotein and undergoes post-translational modification by phosphorylation and glycosylation. Glycosylation may influence the partitioning of IGFBP-3 between the extracellular milieu and the cell surface. While the carbohydrate units appear to be non-essential for the acid-labile subunit or IGF binding, they may modulate other biological activities of IGFBP-3 such as cell-binding activity. If IGFBP-3 can indeed be O-glycosylated, it is necessary to test whether IGFBP-3 is a substrate for GalNAc-T14. The modulation of a new type of glycosylation can extend to the rates of clearance or protease susceptibility of IGFBP-3 present in different physiological and pathological conditions and has an effect on the interaction of IGFBP-3 with other proteins. Hence, it is necessary to explore the relationship between the regulatory mechanism of IGFBP-3 glycosylation and its biological functions (Wu *et al.* 2007b).

In summary, we have shown that interaction between IGFBP-3 and GalNAc-T14 has some functional significance for IGFBP-3-induced apoptosis, and GalNAc-T14 may be involved in the regulation of apoptosis in human RCC 786-O cells. Experiments to determine the mechanism of action of GalNAc-T14 in the regulation of apoptosis induced by IGFBP-3 are under way.

Acknowledgements

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References

Brockhausen I 1999 Pathways of O-glycan biosynthesis in cancer cells; *Biochim. Biophys. Acta.* **1473** 67–95

Delbe J, Blat C, Desauty G and Harel L 1991 Presence of IDF45 (mlGFBP-3) binding sites on chick embryo fibroblasts; *Biochem. Biophys. Res. Commun.* **179** 495–501

Fanayan S, Firth S M, Butt A J and Baxter R C 2000 Growth inhibition by insulin-like growth factor-binding protein-3 in T47D breast cancer cells requires transforming growth factor-beta (TGF-beta) and the type II TGF-beta receptor; *J. Biol. Chem.* **275** 39146–39151

Firth S M and Baxter R C 2002 Cellular actions of the insulin-like growth factor binding proteins; *Endocr. Rev.* **23** 824–854

Gui Y and Murphy L J 2001 Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) binds to fibronectin (FN): demonstration of IGF-I/IGFBP-3/fn ternary complexes in human plasma; *J. Clin. Endocrinol. Metab.* **86** 2104–2110

Huang S S, Ling T Y, Tseng W F, Huang Y H, Tang F M, Leal S M and Huang J S 2003 Cellular growth inhibition by IGFBP-3 and TGF-beta1 requires LRP-1; *FASEB J.* **17** 2068–2081

Kirman I, Poltoratskaia N, Sylla P and Whelan R L 2004 Insulin-like growth factor-binding protein 3 inhibits growth of experimental colocarcinoma; *Surgery* **136** 205–209

Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry T J, Kirschner M W, Koths K, Kwiatkowski D J and Williams L T 1997 Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis; *Science* **278** 294–298

Leal S M, Liu Q, Huang S S and Huang J S 1997 The type V transforming growth factor beta receptor is the putative insulin-like growth factor-binding protein 3 receptor; *J. Biol. Chem.* **272** 20572–20576

Lee H Y, Moon H, Chun K H, Chang Y S, Hassan K, Ji L, Lotan R, Khuri F R and Hong W K 2004 Effects of insulin-like growth factor binding protein-3 and farnesyltransferase inhibitor SCH66336 on Akt expression and apoptosis in non-small-cell lung cancer cells; *J. Natl. Cancer Inst.* **96** 1536–1548

Liu B, Lee H Y, Weinzimer S A, Powell D R, Clifford J L, Kurie J M and Cohen P 2000 Direct functional interactions between insulin-like growth factor-binding protein-3 and retinoid X receptor-alpha regulate transcriptional signaling and apoptosis; *J. Biol. Chem.* **275** 33607–33613

McGuire E J and Roseman S 1967 Enzymatic synthesis of the protein-hexosamine linkage in sheep submaxillary mucin; *J. Biol. Chem.* **242** 3745–3747

Mishra S, Raz A and Murphy L J 2004 Insulin-like growth factor binding protein-3 interacts with autocrine motility factor/phosphoglucose isomerase (AMF/PGI) and inhibits the AMF/PGI function; *Cancer Res.* **64** 2516–2522

Oh Y, Muller H L, Pham H and Rosenfeld R G 1993 Demonstration of receptors for insulin-like growth factor binding protein-3 on Hs578T human breast cancer cells; *J. Biol. Chem.* **268** 26045–26048

Rajah R, Valentinis B and Cohen P 1997 Insulin-like growth factor (IGF)-binding protein-3 induces apoptosis and mediates the effects of transforming growth factor-beta1 on programmed cell death through a p53- and IGF-independent mechanism; *J. Biol. Chem.* **272** 12181–12188

Ricort J M 2004 Insulin-like growth factor binding protein (IGFBP) signalling; *Growth Horm. IGF. Res.* **14** 277–286

Sawada T, Ho J J, Chung Y S, Sowa M and Kim Y S 1994 E-selectin binding by pancreatic tumor cells is inhibited by cancer sera; *Int. J. Cancer.* **57** 901–907

Schedlich L J, O'Han M K, Leong G M and Baxter R C 2004 Insulin-like growth factor binding protein-3 prevents retinoid receptor heterodimerization: implications for retinoic acid-sensitivity in human breast cancer cells; *Biochem. Biophys. Res. Commun.* **314** 83–88

Spagnoli A, Torello M, Nagalla S R, Horton W A, Pattee P, Hwa V, Chiarelli F, Roberts C T, Jr and Rosenfeld R G 2002 Identification of STAT-1 as a molecular target of IGFBP-3 in the process of chondrogenesis; *J. Biol. Chem.* **277** 18860–18867

Wang H, Tachibana K, Zhang Y, Iwasaki H, Kameyama A, Cheng L, Guo J, Hiruma T, Togayachi A, Kudo T, Kikuchi N and Narimatsu H 2003 Cloning and characterization of a novel UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase, pp-GalNAc-T14; *Biochem. Biophys. Res. Commun.* **300** 738–744

Wu C, Wang Y, Zou M, Shan Y, Yao G, Wei P, Chen G, Wang J and Xu D 2007a Prokaryotic expression, purification, and production of polyclonal antibody against human polypeptide N-acetylgalactosaminyltransferase 14; *Protein Expr. Purif.* **56** 1–7

Wu C, Yao G, Zou M, Chen G, Wang M, Liu J, Wang J and Xu D 2007b N-Acetylgalactosaminyltransferase 14, a novel insulin-like growth factor binding protein-3 binding partner; *Biochem. Biophys. Res. Commun.* **357** 360–365

Yamanaka Y, Fowlkes J L, Wilson E M, Rosenfeld R G and Oh Y 1999 Characterization of insulin-like growth factor binding protein-3 (IGFBP-3) binding to human breast cancer cells: kinetics of IGFBP-3 binding and identification of receptor binding domain on the IGFBP-3 molecule; *Endocrinology* **140** 1319–1328

Zappala G, Elbi C, Edwards J, Gorenstein J, Rechler M M and Bhattacharyya N 2008 Induction of apoptosis in human prostate cancer cells by IGF binding protein-3 does not require binding to retinoid X receptor-(alpha); *Endocrinology* **149** 1802–1812

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