Regulation of connective tissue growth factor (CTGF) by hepatocyte growth factor in human tubular epithelial cells

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Abstract

Background. Hepatocyte growth factor (HGF) is a pleiotropic protein with renoprotective functions, which have been attributed at least in part to its ability to counteract the profibrotic effects of transforming growth factor β (TGF-β). A major downstream mediator of TGF-β is connective tissue growth factor (CTGF). However, the molecular mechanisms of CTGF regulation by HGF have not yet been investigated.

Methods. CTGF expression was analysed in human primary tubular epithelial cells (hPTECs) and the cell line HKC-8 by western blotting. Morphological alterations were analysed by immunocytochemistry.

Results. HGF induced a transient expression of CTGF, which was maximal after 6 h and returned to baseline after 24 h. Coincubation with TGF-β increased CTGF protein at 6 h, whereas HGF significantly decreased CTGF induction by TGF-β after 24 h. Furthermore, HGF induced cell scattering associated with reorganization of focal adhesions and formation of lamellipodia and filopodia. The early induction of CTGF was linked to the HGF-mediated alterations of cell morphology. The PP2 inhibitor of Src-family kinases, which regulate focal adhesion turnover, reduced HGF-mediated upregulation of CTGF. In addition, inhibition of the Rho-kinase, which modulates the actin cytoskeleton, impaired CTGF expression. Combination of both inhibitors further decreased CTGF expression. Comparable inhibitory effects were obtained, when CTGF was induced by the combination of HGF and TGF-β.

Conclusions. We provide evidence for a dual effect of HGF on CTGF regulation in human tubular epithelial cells: transient upregulation of CTGF in the absence of TGF-β, which was related to alterations of cell morphology, and interference with TGF-β-mediated CTGF induction after prolonged incubation.

Keywords: actin cytoskeleton; connective tissue growth factor; focal adhesion; hepatocyte growth factor; transforming growth factor β.

Introduction

Hepatocyte growth factor (HGF) is a pleiotropic protein originally isolated as potent mitogen for hepatocytes. The function of HGF, however, is not restricted to the liver. HGF has been recognized as an important regulator of kidney function. Evidence from a variety of models, including remnant kidney, unilateral ureteral obstruction (UUO) and diabetic nephropathy, has demonstrated that HGF ameliorates chronic renal disease (e.g. [1–3]). Upregulation of the HGF receptor c-Met was observed in early acute tubular necrosis, suggesting a role for HGF also in acute renal failure [4]. This was supported by targeting of HGF to the proximal tubule, where it protected from ischaemia-induced acute renal failure by enhancing tubule cell proliferation and decreasing apoptotic cell death [5]. Administration of recombinant HGF in a rat model of acute ischaemic insult to the kidney enhanced the functional and morphological recovery of injured tubular epithelium [6].

In the kidney, HGF is mainly synthesized by interstitial cells, whereas its receptor c-Met is ubiquitously expressed. As a multifunctional cytokine, HGF affects epithelial cell growth and survival, morphogenesis, motility as well as gene expression [7,8].

The beneficial effects of HGF have been attributed at least in part to its ability to counteract the profibrotic effects of transforming growth factor β (TGF-β) [9]. One downstream mediator of TGF-β is the fibrogenic connective tissue growth factor (CTGF). Both TGF-β and CTGF were shown to increase expression of extracellular matrix proteins. In advanced diabetic nephropathy characterized by high TGF-β expression, HGF gene therapy diminished CTGF expression [10], but the molecular mechanisms of CTGF regulation by HGF have not yet been investigated. CTGF is upregulated in vivo and in vitro by TGF-β.
in different renal cells, amongst other tubular epithelial cells (e.g. [11–13]). In murine proximal tubular epithelial cells, CTGF mRNA was transiently upregulated upon incubation with TGF-β [14]. Coincubation with HGF increased the TGF-β-induced CTGF mRNA expression by 20% within the first 8 h, but HGF was not analysed alone and no protein data were provided to appreciate the biological effect of this rather moderate upregulation. At later time points, CTGF expression was decreased rather than increased by coincubation [14]. Similarly, CTGF mRNA levels were reduced, when cells were preincubated with HGF for 24 h prior to stimulation with TGF-β [9]. The effect of HGF alone was assessed in MCT cells and resulted in a moderate increase of CTGF mRNA expression within 24 h [15]. These data suggested that induction or inhibition of CTGF expression is dependent on the cellular context.

A prominent feature of HGF is its ability to alter cell morphology and motility. Most prominently, incubation of Madin-Darby canine kidney (MDCK) epithelial cells with HGF leads to dispersion of the otherwise tightly associated cells [16]. Based on this function, HGF was described as scatter factor. A marked effect on cell morphology was also observed in a rabbit proximal tubular cell line [17], whereas no such data have been reported related to human tubular epithelial cells. Activation of proteins of the family of small Rho GTPases (Rac and Cdc42) as well as their downstream mediators PAK and Rho-kinase (ROCK) has been implicated in the morphological alterations observed in MDCK cells [18,19]. While HGF-mediated alterations of cell morphology are well documented, the functional impact of these alterations on gene expression has not been addressed yet.

Of interest, CTGF expression is regulated by alterations in cell morphology and rearrangement of the actin cytoskeleton [20]. Activation of RhoA/ROCK signalling leads to strengthened Filamentous actin (F-actin) stress fibres that correlate with increased CTGF expression [21,22]. Monomeric G-actin has been shown to be a negative regulator of CTGF gene transcription [23]. Furthermore, rearrangement of focal adhesions modulates CTGF expression in renal fibroblasts, which was linked to the activity of Src-family kinases [24]. Thus far, alterations in cell morphology due to HGF treatment have not been associated with altered gene expression of CTGF.

It was the aim of the present study to analyse the molecular mechanisms of CTGF regulation by HGF in human tubular epithelial cells. We observed HGF-mediated CTGF induction caused by alterations of cell morphology after 6 h, whereas after 24 h HGF interfered with TGF-β-induced CTGF expression.

Materials and methods

Materials

DMEM/Ham’s F12 medium was purchased from Biochrom AG (Berlin, Germany), DMEM medium from PAA Laboratories (Pasching, Austria), fetal calf serum (FCS) from PAN Biotech (Aidenbach, Germany) and insulin-transferrin-selenium supplement from Gibco (Karlsruhe, Germany). HGF was prepared as described [25]. LY-294002 was obtained from Biomol (Hamburg, Germany), Y-27632, PP2 and PP3 from Calbiochem (Merck, Darmstadt, Germany) and TGF-β from Tebu (Frankfurt, Germany). Appropriate solvent controls were used in all experiments.

Cell culture

HKC-8 cells were kindly provided by L. Racusen (Baltimore, MD, USA). Cells were cultured in DMEM/Ham’s F-12 medium 1:1 mixture supplemented with 2.5 % FCS, 2 mM l-glutamine, insulin-transferrin-selenium supplement, 100 U/ml penicillin and 100 μg/ml streptomycin [26]. For experiments, 150 × 10³ HKC-8 cells/10 cm² were seeded in 1% FCS unless otherwise indicated. Human primary tubular epithelial cells (hPTECs) were isolated according to Detrisac et al. [27] (approved by the local ethics committee). In brief, healthy renal cortical tissues were collected from tumour nephrectomies and transported in cooled Hank’s balanced salt solution HBSS. The outer cortex was cut into 1 mm³ pieces that were cultured in a serum-free medium used for HKC-8 cells, also containing 10 ng/ml epidermal growth factor, 36 ng/ml hydrocortisone and 4 pg/ml triiodothyronine. On sufficient outgrowth, cells were subcultured by application of trypsin. Experiments were conducted on passage 3 and 4 (day 20–30). MDCK cells were cultured in DMEM low glucose supplemented with 10% FCS, 2 mM l-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. For experiments, subconfluent cultures were used. After serum starvation for 24 h, HGF was added unless otherwise indicated. Kinase inhibitors were added 30 min prior to HGF or TGF-β.

Scatter assay

MDCK and HKC-8 cells were seeded in FCS-containing medium in 6-well plates. After 3 h, the medium was changed to 0% FCS and HGF was added. Cells were incubated overnight, and cell morphology was recorded by an Olympus CK40 microscope (Olympus, Hamburg, Germany) using Leica DC Viewer software (Leica, Herbrugg, Switzerland).

Western blot analysis: Cells were lyzed in a buffer containing 9 M urea, 2% CHAPS, 1 mM DTT, 2 mM sodium vanadate and protease inhibitors complete EDTA-free (Roche diagnostics, Basel, CH, Switzerland). Proteins were separated by 12% SDS-PAGE and transferred onto PVDF membranes (Macherey-Nagel, Düren, Germany). The following antibodies were used: goat polyclonal anti-CTGF (SC-14939, Santa Cruz, Heidelberg, Germany), rabbit polyclonal anti-phospho-AKT (#9271, Cell Signalling, Danvers, MA, USA), rabbit polyclonal anti-AKT (SC-8312, Santa Cruz), mouse monoclonal anti-tubulin (E7, Developmental Studies Hybridoma Bank, University of Iowa, LA, USA; developed by Klymkowsky), HRP-conjugated donkey anti-goat IgG (SC-2020, Santa Cruz), HRP-conjugated sheep anti-mouse IgG and donkey anti-rabbit IgG (Amersham Biosciences, Freiburg, Germany). Immunoreactive proteins were visualized by the enhanced...
chemiluminescence detection system (ECL-Plus, Amersham Biosciences). Bands were quantified using the luminescent image analyser (LAS-1000 Image Analyzer, Fujifilm, Berlin, Germany) and AIDA 4.15 image analyser software (Raytest, Berlin, Germany). To correct for equal loading and blotting, all blots were redetected with antibodies directed against tubulin or AKT. For quantification purposes, the ratio of the specific protein band and a control protein was calculated.

**Immunocytochemistry**

Cells were fixed with paraformaldehyde (3.5% in PBS) for 10 min and afterwards permeabilized by 0.2% Triton X-100 in PBS for 10 min. After washing three times with PBS, cultures were blocked in 5% horse serum in PBS for 1 h at room temperature. Cells were incubated with an anti-phosphotyrosine antibody (#9411, Cell Signalling; 1:100 in PBS) overnight at 4°C and with a secondary Alexa Fluor 488-conjugated anti-mouse antibody (Invitrogen, Eugene, OR, USA; 1:500 in PBS) for 45 min. F-actin was visualized by phalloidin FluoProbes 547 staining (Interchim, Montlucon, France). After mounting, slides were viewed using a Nikon fluorescent microscope and digital images recorded using EclipseNet imaging software (Laboratory Imaging, Praha, Czechoslovakia). Staining was repeated at least two times.

**Statistical analysis**

To compare multiple conditions, statistical significance was calculated by one-way ANOVA with Tukey–Kramer multiple comparison *post hoc* tests. A value of $P < 0.05$ was considered to indicate significance.

**Results**

*HGF transiently induces CTGF expression in human tubular epithelial cells*

To study the effects of HGF on the regulation of CTGF expression, we used hPTECs isolated from healthy cortex tissue of tumour nephrectomies. Stimulation with 5 ng/ml HGF for 6 h led to a slight but significant induction of CTGF that was reproducible in different preparations of hPTECs (Figure 1A). This induction was transient, as CTGF expression returned to baseline after 24 and 48 h. The same effect of HGF on CTGF expression was also observed in the human proximal tubular cell line HKC-8 (Figure 1B) that showed increased CTGF expression at 2, 6 and 12 h and basal expression at 24 and 48 h.

*PI3K/AKT signalling is not involved in CTGF induction by HGF*

HKC-8 cells were used for the further analysis of the molecular mechanisms underlying the transient HGF-mediated upregulation of CTGF. Ishibe *et al.* demonstrated in mouse inner medullary duct cells that high cell density can specifically downregulate PI3K/AKT signalling that promotes HGF-regulated morphogenesis [28]. In line with these data, basal levels of phosho-AKT (pAKT) were high in sparse HKC-8 cells and reduced in high density cells (Figure 2A). Like pAKT, expression of CTGF decreased with cell density (Figure 2A). Upon treatment with HGF, phosphorylation of AKT was induced at all cell densities (fold induction low density: 2.2 ± 0.2; medium density: 1.9 ± 0.5; high density: 2.8 ± 0.1; means ± SD, $n = 3$) that was paralleled by increased CTGF expression (fold induction low density: 2.1 ± 0.2; medium density: 2.5 ± 0.2; high density: 2.3 ± 0.4; means ± SD, $n = 3$). These findings suggested a correlation between AKT activity and CTGF expression. However, inhibition of PI3K/AKT signalling by the specific PI3K inhibitor LY-294002 did not interfere with HGF-mediated CTGF upregulation (Figure 2B), while preincubation of HKC-8 cells with the inhibitor completely prevented AKT phosphorylation (Figure 2C). These data indicated that HGF-mediated induction of CTGF expression was not linked to PI3K/AKT signalling.

*HGF induces morphological alterations in human tubular epithelial cells*

We have previously shown that the expression of CTGF is regulated by alterations of the cytoskeleton in renal cells.
Fig. 2. CTGF induction by HGF is independent of PI3K/AKT signalling. (A) HKC-8 cells were plated with low (75 × 10^3 cells/10 cm^2), medium (150 × 10^3 cells/10 cm^2) and high cell density (300 × 10^3 cells/10 cm^2) and were treated with 5 ng/ml HGF for 6 h. A representative blot analysing CTGF, pAKT and AKT expression is shown (n = 3). (B) After preincubation with the PI3K inhibitor LY-294002 (LY, 10 µM) for 30 min, HKC-8 cells (150 × 10^3 cells/10 cm^2) were stimulated with HGF (5 ng/ml) for 6 h. The graph displays the statistical analysis of CTGF expression of four independent experiments. For normalization, CTGF expression of HGF-treated cells was set to 100%; data are means ± SD. (C) HKC-8 cells were preincubated with 10 µM LY-294002 for 30 min and then stimulated with HGF for 6 h. pAKT and AKT were detected by western blotting.

[21,23,24]. Therefore, we analysed the effect of HGF on cell morphology. The scattering activity of HGF in the canine tubular cell line MDCK is well established. Therefore, these cells were used in comparison with the human HKC-8 cells, the behaviour of which has not been described yet. Under control conditions, MDCK cells formed characteristic cobblestone-like cell clusters that were less pronounced in HKC-8 cells (Figure 3 left panel). Incubation with 10 ng/ml HGF caused cell scattering associated with formation of cell extensions in both cell lines (Figure 3 right panel). These effects were also obvious, but weaker at 5 ng/ml HGF.

To detect more subtle changes in cell morphology, we analysed the expression of focal adhesions and the actin cytoskeleton in HKC-8 cells. Adherent cells are attached to the extracellular matrix via focal adhesion complexes that undergo dynamic rearrangements. As a marker of focal adhesion alterations, phospho-tyrosine (pTyr) was detected by immunocytochemistry. Under basal conditions, focal adhesions were evenly distributed in HKC-8 cells (Figure 4A). Treatment with 5 ng/ml HGF for 30 min triggered a redistribution of pTyr to the cell periphery (Figure 4B). The actin cytoskeleton of HKC-8 cells is characterized by strong cell spanning F-actin stress fibres and cortical actin fibres (Figure 4C). Upon HGF treatment (5 ng/ml for 30 min), only minor changes of actin fibres were detectable in confluent HKC-8 cells. However, there were distinct alterations in cells at the monolayer boundaries (Figure 4D). Cortical actin fibres were reduced, and lamellipodia and filopodia were found indicative of an activation of the small GTPases Rac and Cdc42 [29].

Changes in cell morphology are related to CTGF expression

Next, we investigated whether the HGF-induced morphological alterations were related to the enhanced CTGF

Fig. 3. HGF induces cell scattering in HKC-8 cells. HKC-8 and MDCK cells were seeded for 3 h to settle, subsequently serum starved and treated with HGF overnight. Bright field pictures were recorded. Representative pictures for 10 ng/ml HGF are shown. Scale bar: 100 µm.

Fig. 4. Morphological alterations are induced by HGF in HKC-8 cells. HKC-8 cells were seeded on fibronectin-coated glass coverslips and allowed to settle overnight. After serum starvation for 24 h, cells were stimulated with 5 ng/ml HGF for 30 min. Cells were stained for phospho-tyrosine as a marker for focal adhesions by indirect immunocytochemistry (A and B), and F-actin was visualized by phalloidin staining (C and D). Images are representative for three independent experiments. Scale bar: 25 µm.
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Fig. 5. CTGF induction by HGF is mediated by Src-family kinases and ROCK. (A) HKC-8 cells were preincubated with the Src-family kinase inhibitor PP2 (5 \( \mu \)M), the inactive PP2 analogue PP3 (5 \( \mu \)M) for 30 min as indicated, and stimulated with 5 ng/ml HGF for 6 h. CTGF was detected by western blot analysis. The graph summarizes the results of six independent experiments; data are means \( \pm \) SD; \( \ast\ast\ast \) \( P \) < 0.001 compared to HGF-treated control cells. (B) HKC-8 cells were preincubated with the ROCK inhibitor Y-27632 (10 \( \mu \)M) and then stimulated with 5 ng/ml HGF for 6 h. A representative western blot is presented. Quantification of the inhibitory effect is included in Fig. 6B.

expression. Based on alterations of focal adhesions in HKC-8 cells upon incubation with HGF (Figure 4), we hypothesized a role for Src-family kinase signalling in the transient induction of CTGF in human tubular cells, as this signalling pathway regulates focal adhesion turnover [30]. Treatment of HKC-8 cells with the inhibitor of Src-family kinases PP2 (5 \( \mu \)M) significantly inhibited HGF-induced expression of CTGF, while the inactive PP3 analogue had no effect (Figure 5A). Activation of ROCK by HGF has been reported [18], and this kinase has been shown earlier to regulate CTGF expression (e.g. [21]). Therefore, HKC-8 cells were pretreated with the inhibitor Y-27632 (10 \( \mu \)M) and then stimulated with HGF. A partial but significant reduction of HGF-induced CTGF expression was detected (Figure 5B, quantification Figure 6B). These data indicate that Src-family kinases and ROCK modulate HGF-induced CTGF expression.

In tubular epithelial cells, CTGF is a well-known target gene of TGF-\( \beta \), which is the main mediator of renal fibrosis [31]. Thus, the regulation of CTGF expression by HGF in combination with TGF-\( \beta \) was analysed. After 6-h incubation, HGF and TGF-\( \beta \) induced a comparable increase in CTGF expression (Figure 6A). In combination, both growth factors were additive. Compared to the results obtained for HGF, TGF-\( \beta \)-induced CTGF expression was reduced, when the cells were pretreated with PP2 or Y-27632 (Figure 6B), whereas the inactive control compound PP3 did not interfere with TGF-\( \beta \)-mediated upregulation of CTGF (data not shown). The most prominent effect was obtained with a combination of both inhibitors (Figure 6B). Comparable inhibitory effects were also detectable, when HKC-8 cells were coincubated with HGF and TGF-\( \beta \) (Figure 6B).

HGF reduces TGF-\( \beta \)-mediated induction of CTGF upon prolonged incubation

In contrast to the transient upregulation by HGF, TGF-\( \beta \) induced a gradient increase in CTGF protein expression after 6 (1.8 \( \pm \) 0.3 fold) and 24 h (2.9 \( \pm \) 0.3 fold) in HKC-8 cells (Figure 6A and Figure 7). At variance to the additive
Effect of HGF and TGF-β after 6 h, coincubation of the cells with TGF-β and HGF for 24 h led to a reduced expression of CTGF in comparison to TGF-β alone. HGF interference with TGF-β-induced CTGF expression was also clearly seen after 24 h in primary cultures of human tubular epithelial cells of different donors (Figure 7).

**Discussion**

We provide data that show a dual regulation of CTGF by HGF in human tubular epithelial cells: early upregulation due to alterations of cell morphology, and subsequent inhibition due to interference with TGF-β signalling.

HGF induces morphological alterations in epithelial cells, best characterized by its scattering activity in MDCK cells. The human tubular cell line HKC-8 does not cluster as strongly as MDCK cells, and therefore the scattering effect of HGF was less prominent. A detailed analysis of HGF-mediated morphological alterations in HKC-8 cells revealed a reorganization of focal contacts that located along sites of cell–cell contacts. Furthermore, structural changes of the actin cytoskeleton, most notably formation of lamellipodia and filopodia, were observed upon HGF treatment. These alterations were in line with the well-documented activation of the small GTPases Rac and Cdc42 by HGF (e.g. [18,19]).

In most cells, induction of CTGF seems to be regulated by RhoA/ROCK signalling and subsequent alterations of the actin cytoskeleton [20]. In a recent study, Black and Trackman showed in gingival fibroblasts that TGF-β-mediated CTGF induction is Rac dependent and that this increase in CTGF expression was inhibited by the ROCK inhibitor Y-27632 [32]. Additionally, activation of ROCK was detected in response to HGF treatment in epithelial cells besides activation of Rac-1 [18]. While our data clearly showed an inhibitory effect of Y-27632 on CTGF expression induced not only by HGF but also by TGF-β and the combination of both growth factors, the exact role of ROCK and its relationship to other signalling pathways activated by HGF and TGF-β in epithelial cells needs further investigation.

The most prominent alteration induced by HGF treatment was the dissipation of focal contacts in HKC-8 cells. In an earlier study, we reported that morphological manipulation of human renal fibroblasts upregulates CTGF expression that was dependent on strong rearrangements of focal contacts regulated by Src-family kinases when the cells were cultured in/on collagen gels, but not in cells cultured on rigid surfaces [24]. In line with these findings, inhibition of Src-family kinases strongly inhibited HGF-induced CTGF expression in HKC-8 cells, indicating a link between dynamics of focal adhesion organization and CTGF expression. Furthermore, Src-family kinases were also involved in TGF-β-mediated upregulation of CTGF. There is increasing evidence that besides the activation of Smad transcription factors, TGF-β-mediated alterations of cell morphology contribute to changes in gene expression [33,34]. Taken together, our findings implicate that upregulation of CTGF by HGF is dependent on cell–extra cellular matrix interactions.

Cell confluence may reflect tubular epithelial pathophysiology *in vitro*. While dense monolayers of tubular cells correspond to intact tubuli, sparse cells may rather resemble injured tissue. CTGF expression was reduced in dense HKC-8 cells consistent with *in vivo* findings, where CTGF is hardly detectable in healthy kidneys. Cell density was also reflected by the activity of AKT, a downstream mediator of the HGF receptor c-Met. In HKC-8 cells, this pathway has been implicated in the inhibition of the chemokine RANTES by HGF [35]. Furthermore, AKT signalling plays a role in the anti-apoptotic signalling of HGF in HKC-8 cells, where inactivation of the pro-apoptotic mediator BAD and induction of the anti-apoptotic protein Bcl-xl are dependent on AKT signalling [36]. Cell confluency has been shown to regulate HGF signalling in a β-catenin-dependent manner via PI3K/AKT [28] and activation of β-catenin links alterations of cell morphology and gene expression [37]. In our studies, we observed an apparent correlation between cell density-dependent pAKT and CTGF expression, which suggested a link between AKT signalling and CTGF. However, inhibition of HGF-activated PI3K/AKT signalling did not alter CTGF expression, excluding a role for AKT signalling in CTGF induction.

In HKC-8 cells, we show a biphasic regulation of CTGF protein expression by coincubation with HGF and TGF-β. A similar effect was observed by Inoue *et al.* [14] for the regulation of CTGF mRNA expression. At later time points, TGF-β-induced CTGF expression was inhibited by HGF. HGF has been shown to induce cell type-specific proteins that interfere with TGF-β signalling. In mesangial cells, HGF stabilizes the Smad transcriptional corepressor TGIF [38]. In HKC-8 epithelial cells, the Smad transcriptional corepressor SnoN was shown to be a mediator of the antagonistic interaction of HGF and TGF-β, whereas TGIF expression was barely detectable in these cells [39]. Upregulation of SnoN was also related to the antifibrotic effects of HGF in the UUO model [40]. This suggests that SnoN may be involved in the regulation of CTGF expression after prolonged incubation of epithelial cells with HGF, which needs to be investigated by further experiments.

Taken together, our data provide evidence for a biphasic regulation of CTGF by HGF in human tubular epithelial
cells. While the early induction of CTGF was dependent on cell morphology regulated by Src-family kinases and ROCK, interference with TGF-β-mediated induction of CTGF was detectable in both hPTECs and HKC-8 cells at later time points. These data are concordant with animal experiments that relate the renoprotective effect of HGF to the inhibition of TGF-β signalling, not excluding other protective effects of the pleiotropic factor HGF.

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Conflict of interest statement. None declared.

References


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