Expression of subtypes of somatostatin receptors in hepatic stellate cells

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AIM: To elucidate the mechanism by which somatostatin and its analogue exert the influence on liver fibrosis, and to investigate the mRNA expression of somatostatin receptors subtypes (SSTRs) and the distribution of somatostatin analogue octreotide in rat hepatic stellate cells (HSCs).

METHODS: HSCs were isolated from Sprague Dawley (SD) rats by in situ perfusion and density gradient centrifugation. After several passages, the mRNA expression of 5 subtypes of SSTRs were assessed by reverse transcription-polymerase chain reaction (RT-PCR). HSCs were planted on coverslip and co-cultured with octreotide tagged by FITC. Then the distribution of FITC fluorescence was observed under laser scanning confocal microscope (LSCM) in 12-24 h.

RESULTS: There were mRNA expression of SSTR2, SSTR3 and SSTR5 but not SSTR1 and SSTR4 in SD rat HSCs. The mRNA expression level of SSTR2 was significantly higher than that of other subtypes (P<0.01). FITC fluorescence of octreotide was clearly observed on the surface and in the cytoplasm, but not in the nuclei of HSCs under LSCM.

CONCLUSION: The effect exerted by somatostatin and its analogues on regulating the characters of active HSCs. This provides a potential prevention and management against liver fibrosis.

Isolation and culture of hepatic stellate cells (HSC)

Male Sprague Dawley rats (450±50 g) were purchased from Experimental Animal Center of the Chinese Academy of Medical Science, Beijing, China. Nycodenz, fluorescein isothiacyanate (FITC) and collagenase IV were purchased from Sigma Co., U.S.A. Fetal bovine serum (FBS), Dulbecco MEM (DMEM) culture medium, Trizol and superscript II reverse transcriptase were produced by Gibco Co., U.S.A. Taq DNA polymerase, dNTPs, Oligo (dT) and RNasin were products of Sangon Co. Mouse anti-human desmin antibody and rabbit anti-cow isothiacyanate (FITC) and collagenase IV were purchased from Genetech Co. Primers were synthesized by Shanghai Sangon Co.

Isolation and culture of hepatic stellate cells (HSC)

Adult male Sprague Dawley rats (450±50 g) were treated in accordance with the institution’s guidelines for the care and use of laboratory animals in research. All procedures were performed with animals under ether anesthesia. According to the method of Geerts and Weng et al[10,11], HSCs were isolated by in situ perfusion and density gradient centrifugation with nycodenz. Firstly, the trochar was inserted into the rat’s portal vein and the liver was perfused with pre-perfusion liquid. Then the liver was removed from the body and continuously perfused with perfusion liquid containing collagenase IV to digest the extracellular matrix. After centrifugation with 150 g/L nycodenz, the cells located in the interface were extracted and cultured in DMEM containing 200 mL/L FBS. HSC were fully stick to the wall within 48 h. Then the culture medium was exchanged in every 2-3 d. The identification of HSC was performed by observation of ultraviolet-excited fluorescence of vitamine A
lipid droplet in HSC and immunocytochemical stain for desmin and GFAP.

**Isolation of total RNA and semiquantitative RT-PCR**

Total RNA was isolated from HSC with Trizol by phenol-chloroform extraction and isopropanol precipitation following manufacturer’s instructions. Reverse transcription of total RNA was carried out according to the instructions of the RT kit. Design of specific primers against rat SSTRs sequences was referred to the references and verified in NCBI Blast. They are shown in Table 1. The PCR system (total 25 µL) contained 40 pmol/L primers of SSTRs or β-actin, 0.5 µL of 10 mmol/L dNTPs, 1.5 µL of 50 mmol/L MgCl2, 2 µL of cDNA, 2.5 µL of 10×PCR buffer, 2.5 µL of Taq DNA polymerase. The PCR conditions included an initial denaturation for 5 min at 94°C; 40 amplification cycles consisting of denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min and elongation at 72°C for 1 min; and a final extension at 72°C for 7 min. The PCR products or DNA marker mixed with 2 µL of loading buffer were electrophoresed on a 15 g/L agarose gel and visualized under ultraviolet light excitation. Then the images were taken and analyzed by Kodak digital camera and science software (Kodak, EDAS290, U.S.A). The expression of SSTRs was calculated by determining the ratio of SSTRs relative to β-actin.

**Table 1** Primer sequence and expected PCR product length

| Primer designation | Sequence | Product length |
|--------------------|----------|---------------|
| SSTR1 upstream     | 5'-CAC GCA CCG CAG CCA ACA-3' | 390 bp |
| SSTR1 downstream   | 5'-GGA AGC CGT AGA GTA TGG GGT T-3' | 390 bp |
| SSTR2 upstream     | 5'-CCG GAG CAA CCA GTG GGG-3' | 390 bp |
| SSTR2 downstream   | 5'-GCG TAC AGG ATG GGG TTG GC-3' | 390 bp |
| SSTR3 upstream     | 5'-CCC GGG GCA TGA GCA CGT-3' | 415 bp |
| SSTR3 downstream   | 5'-AAG CCG TAG AGG ATG GGG TTT GC-3' | 415 bp |
| SSTR4 upstream     | 5'-TCG TGG GGG TGA GGC AGT AG-3' | 365 bp |
| SSTR4 downstream   | 5'-CAT AGA GAA TCG GGG TGG GAC A-3' | 365 bp |
| SSTR5 upstream     | 5'-ATG GAG CCC CTC TCT CTG G-3' | 250 bp |
| SSTR5 downstream   | 5'-CGT CAG CCA CGG CCA GGT T-3' | 250 bp |
| β-actin upstream   | 5'-TGG GAC GAT AGT GAG GAA AT-3' | 522 bp |
| β-actin downstream | 5'-ATT GCC GAT AGT GAT GAC CT-3' | 522 bp |

The observation of combination of octreotide with HSC and distribution in HSC

After 3-4 passages, HSCs were planted on coverslip and culture medium was exchanged after sticking to the walls. Then octreotide tagged by FITC was added into the culture medium (10 µg/mL). After 24 h, the culture medium was removed and the coverslips were rinsed three times with PBS, wherein the distribution of FITC fluorescence in live HSCs was observed by laser scanning confocal microscope (LSCM).

**Statistical methods**

The results of electrophoresis of PCR products were expressed as mean±SD and analyzed by t-test and analysis of variance.

**RESULTS**

**Expression of mRNA of somatostatin receptor subtypes**

The mRNA expression of SSTR2, SSTR3 and SSTR5 were observed, whereas those of SSTR1 and SSTR4 were not observed in rat HSCs. Moreover the mRNA expression level of SSTR2 was significantly higher than those of other subtypes (4 and 5.64 times higher than that of SSTR3 and SSTR5, respectively, P<0.01). There was no significant difference between the mRNA expression of SSTR3 and SSTR5 (Figures 1, 2, and 3, Table 2).

**Table 2** Expression of SSTRs mRNA in quantity (mean±SD)

| Subtypes | n | Expression of mRNA |
|----------|---|-------------------|
| SSTR2    | 3 | 0.821±0.1210      |
| SSTR3    | 3 | 0.204±0.1662      |
| SSTR5    | 3 | 0.145±0.1981      |

β-actin 522 bp
SSTR2 390 bp

![Figure 1](image1.png)

Expression of SSTR2 mRNA. M: 100 bp Marker; Lanes 1-3: three groups of samples.

SSTR3 415 bp

![Figure 2](image2.png)

Expression of SSTR3 mRNA. M: 100 bp Marker; Lanes 1-3: three groups of samples.

SSTR5 250 bp

3 µm

![Figure 3](image3.png)

Expression of SSTR5 mRNA. M: 100 bp Marker; Lanes 1-3: three groups of samples.

**Distribution of octreotide in HSCs**

The green fluorescence of FITC had an extensive distribution on the surface of HSCs and also in cytoplasm, but not in nucleus (Figure 4).

![Figure 4](image4.png)

Distribution of the green fluorescence of octreotide tagged by FITC in live HSCs. FITC fluorescence of octreotide was clearly observed on the surface and in the cytoplasm, but not in the nuclei of HSCs under LSCM.
Several studies have demonstrated that somatostatin could restrain and decrease the process of hepatic fibrosis of rat liver cirrhosis models\(^{[4-6]}\). In addition, researchers studied the anti-fibrosis mechanisms of somatostatin. Reynaert et al.\(^{[13]}\) proved that somatostatin could suppress rat hepatic stellate cell contraction induced by endothelin-1. Chatterjee et al.\(^{[17]}\) found that in rat liver cirrhosis models of schistosomiasis, somatostatin might have the direct anti-fibrosis effects through modulating the synthesis and expression of collagen I, III and smooth muscle actin (SMA) of rat HSCs. All of these findings suggest that there are SSTRs on the surface of HSCs, thereby somatostatin could exert its actions on HSCs.

Whether SSTRs exist and what kind of SSTRs subtypes are expressed in rat HSCs? We observed mRNA expression of SSTR2, SSTR3 and SSTR5, but not of SSTR1 and SSTR4 in Sprague Dawley rat HSCs. Our results slightly differed from Reynaert et al.\(^{[13]}\) that SSTR1, SSTR2 and SSTR3 were expressed, but not SSTR4 and SSTR5 in Wistar rat HSCs. Besides, we found that the mRNA level of SSTR2 was significantly higher than those of other subtypes in quantity. These results suggest that theoretically, somatostatin may be available to exert its inhibitory and pre-apoptosis actions on active HSCs by means of SSTRs (especially SSTR2) and consequently has the ability of anti-fibrotic action. Therefore, if the proper somatostatin analogues that have strong affinity to SSTR2, 3 and 5 are chosen, they will be able to exert better effect on active HSCs.

Taking advantage of the strong appetency by which somatostatin could combine with SSTRs, some researchers have applied the somatostatin analogues tagged by radioactive isotope to study the expression and distribution of SSTRs in cancer tissues and consequently the locational diagnosis of cancers. This kind of method has been proved to be satisfactory. Since our results showed that SSTR2, 3 and 5 were expressed and other studies reported that octreotide had the special affinity to SSTR2 and SSTR5\(^{[7,9]}\), we treated HSCs with octreotide tagged by FITC. By means of the characteristics that hormones can specially bind to its receptors, we observed the green fluorescence on the surface of live HSCs, which reflected the distribution of SSTRs. This proves that octreotide has strong affinity with HSCs and thus the existence of SSTRs is supported. Meanwhile, we also observed the distribution of octreotide in the cytoplasm, but not in the nuclei of HSCs. In general, octreotide contains 8 peptides, so it can’t pass through the cell membrane without other pathways were introduced. Thereby, we presume that the endocytosis conducted by the receptors may works. But whether octreotide in cytoplasm is able to exert its biological actions by a certain mechanism independent of SSTRs remains to be confirmed in the future.

In conclusion, we demonstrates that SSTR2, 3 and 5 are obviously expressed in SD rat HSCs. In addition, octreotide, a somatostatin analogue, is able to combine perfectly with HSCs and thereby exerts its biological actions on liver fibrosis in clinical practice.

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