Transforming growth factor β-like activity in human hydrocele fluid

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Summary. In this study transforming growth factor β (TGF-β)-like activity in human hydrocele fluid was investigated. Inhibition of DNA synthesis of adult rat hepatocytes in primary culture and stimulation of colony formation of normal rat kidney (NRK) fibroblasts, clone 49F in soft agar were observed in all acidified hydrocele fluids and these activities were neutralized by the specific antibody raised against human native TGF-β. In samples obtained from recurrent cases of hydrocele, TGFβ-like activity was observed in its active form (without acidification). These results suggest that human hydrocele fluid contains TGFβ-like activity and that the active form of TGF-β in recurrent hydrocele fluid may be responsible for the recurrence of the disease even after repeated aspiration.

Key words: Transforming growth factor – Hydrocele

TGF-β is a well-known bifunctional regulator of cell growth or cell differentiation [21]. It stimulates the anchorage-independent growth of mouse AKR-2B cells or NRK-49F cells [9] and inhibits the DNA synthesis of rat hepatocytes in primary culture [1, 11], mink lung cells [10] and vascular endothelium [14]. TGF-β was initially isolated from a culture medium conditioned by virally transformed cells [2]. Recently it has been found in many normal and malignant tissues [17]. TGF-β activity has been demonstrated in the malignant effusions of various cancerous patients [20], urine from normal, pregnant and tumor-bearing humans [23] and recently in synovial effusions [3]. TGF-β is usually secreted from the producer cells in a biologically inactive form, which can be activated by transient acidification [15, 24], urea treatment [8] or alkali treatment [6].

While hydrocele is a very common disorder, and recurrence after repeated aspiration is also commonly seen, the mechanism of recurrence is still not clear. We studied human hydrocele fluid in order to determine whether or not it contained TGFβ-like activity using the following four criteria: (i) activation with acidification (ii) inhibition of DNA synthesis of cultured rat hepatocytes (iii) stimulation of colony formation of NRK-49F cells in soft agar and (iv) neutralization of the activity by a specific antibody to TGF-β. Our results indicate that TGFβ-like activity is present in all cases of hydrocele fluid irrespective of age. In addition, our results also demonstrate that only the fluid of recurrent cases contains the active form of TGF-β.

Materials and methods

Williams medium E was obtained from Flow Laboratories, U. K. Dulbecco’s modified Eagle’s medium was from Nissui Pharmaceuticals, Tokyo, Japan. Newborn calf serum was from GIBCO Oriental, Tokyo, Japan. Insulin and bovine serum albumin were purchased from Sigma Chemical Co. Aprotinin was from Mochida Pharmaceuticals, Tokyo, Japan. Dexamethasone and collagenase were from Wako Pure Chemical Industries, Osaka, Japan. 5-[125I]-iododeoxyuridine (2200 Ci/m mol) was from New England Nuclear Boston, MA, USA. Anti-TGF-β antibody was obtained from R&D Systems, USA. Epidermal growth factor (EGF) was purified in our laboratory from the submaxillary glands of adult male mice using the method of Savage and Cohen [19]. Male Wistar rats were obtained from Otsu Experimental Animals, Nagasaki, Japan.

Collection and acid treatment of hydrocele fluid

Fluids were collected from different patients by needle aspiration into disposable plastic syringes. These fluids were routinely centrifuged at 3,000 rpm for 10 min to remove the floating cells and stored at -20°C until used. Acid treatment of the fluid was carried out by the addition of acetic acid (final concentration 1 M) for 6 h at room temperature.

Isolation of rat hepatocytes

Parenchymal hepatocytes were isolated from adult male Wistar rats weighing 150–250 g by two-step collagenase perfusion as previously reported by Tanaka et al [22].
**Fig. 1.** Effect of human hydrocele fluid on DNA synthesis of adult rat hepatocytes in primary culture. Experimental conditions were as described in *Materials and methods*. Values are means of duplicate dishes. ○ = Untreated hydrocele fluid; ● = acid-treated hydrocele fluid

**Fig. 2.** Dose-dependent effects of acid-treated hydrocele fluid on DNA synthesis of adult rat hepatocytes in primary culture and colony formation of NRK-49F cells in soft agar. ○ = Colony formation of NRK-49F cells in soft agar; ● = DNA synthesis of hepatocytes

**Assay of DNA synthesis**

Isolated hepatocytes were suspended in Williams medium E supplemented with 5% newborn calf serum, 10^{-9} M insulin and 10^{-8} M dexamethasone and were incubated into 24-well multi-well plates coated with type 1 collagen at a density of 6.25 × 10^6 cells/cm^2. After 24 h, the medium was changed to a serum and hormone free medium containing 5 U/ml aprotinin, 10^{-7} M insulin and 10 ng/ml EGF. Untreated hydrocele fluid or neutralized activated fluid was then added to the cells. Incorporation of 5-[125I]-iododeoxyuridine into hepatocytes was measured using the procedure described by Nakamura et al. [13].

**Soft agar colony formation assay of NRK-49F cells**

NRK-49F fibroblasts, obtained from the Japanese Cancer Research Resources Bank, were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Colony formation in soft agar was assayed in the presence of EGF as previously described [4, 11].

**Neutralization of TGF β-like activity**

Acidified hydrocele fluid was neutralized with sodium hydroxide and diluted with the culture medium containing 0.5% bovine serum albumin. Anti-human TGF-β IgG was then added and the fluid incubated for 2 h at 37° C. Finally, the fluid was added to the hepatocytes and DNA synthesis was assayed.

**Results**

Figure 1 shows the typical pattern of the inhibition of DNA synthesis of cultured rat hepatocytes by human hydrocele fluid. The acid-treated hydrocele fluid causes dose-dependent inhibition of DNA synthesis of rat hepatocytes stimulated by insulin and EGF, but untreated fluid does not show any activity (Fig. 1). This suggests that the inhibitory activity for DNA synthesis of rat hepatocytes is activated by acid treatment. It has been reported that not only TGF-β but also PDGF-α [12], interleukin-1β [13] and interleukin-6 [13] can suppress the DNA synthesis of adult rat hepatocytes. Of the four, only TGF-β stimulated colony formation of NRK-49F cells in soft agar. To confirm that this inhibitory effect on the growth of hepatocytes was due to the TGF-β-like activity, the acid activated hydrocele fluid was examined for its capacity to promote soft agar colony formation of NRK-49F cells (Fig. 2). Each of the fluids examined was found to promote soft agar colony formation by indicator cells in the presence of EGF.

Further, we studied the effect of anti-human TGF-β IgG on the inhibitory activity of hydrocele fluid on DNA synthesis of cultured rat hepatocytes. Table 1 shows that 40 μg/ml of specific antibody completely neutralized the TGFβ-like activity of hydrocele fluid. These results indicated that human hydrocele fluid possessed typical TGFβ-like activity. But the significance of the TGFβ-like activity in human hydrocele fluid was unclear. Next we screened the activity in hydrocele fluids using cultured rat hepatocytes. Table 2 shows a summary of the screening. All fluids obtained from recurrent cases were found to contain both the active and latent forms of TGFβ-like activity, while nonrecurrent fluids contained only the latent form of TGFβ-like activity. In recurrent cases (Table 2), we used the recurrent samples. Several samples collected from each case during the course of recurrence