IS SERUM-DERIVED HYALURONATE-BINDING PROTEIN, SHAP A DIFFERENT MOLECULAR SPECIES FROM THE HYALURONATE RECEPTOR, GP85?

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We found that proteins (Mr 85000 and 83000) are firmly but not covalently associated with hyaluronate (HA) purified from the cell layer of cultured mouse dermal fibroblasts (Fig. 2, lane d). Their binding to hyaluronate was neither affected by treatment with any type of detergent at high concentrations (ex. 8 % Zwittergent, 0.2 % SDS), nor with various protein-denaturing reagents (ex. 6 M GuHCl, 7 M urea). We then found that these proteins were derived from the fetal calf serum added to culture medium as a supplement (1). Therefore, we have named the protein a serum-derived hyaluronate-binding protein, SHAP.

In both the apparent size and the ability to bind to hyaluronate, SHAP is quite similar to gp85 which was found originally to be the antigen molecule for a monoclonal antibody, K3 by Tarone et al. (2), and subsequently has been shown to be a HA receptor by Underhill et al. (3). We therefore wondered whether SHAP was a molecule identical to gp85 or not. Our preliminary studies (1) have shown tentatively that SHAP is a different molecule from gp85. In this report, we have examined their distributions on cultured cells and reactivities with their specific antibodies. The results have confirmed that SHAP is a different molecule from gp85.

MATERIAL AND METHODS

Mouse dermal fibroblasts were prepared from 3-5-day-old neonates of DDY mice as previously described (1). BHK cells were kindly provided by Dr. R. Ishida, Aichi Cancer Research Center, Nagoya. Both cells were grown to subconfluent monolayers on 12-mm glass coverslips in Dulbecco's modified Eagle's medium with 10 % (v/v) fetal calf serum. Cell layers were fixed with 3 % (w/v) formaldehyde in PBS, 1 % (w/v) sucrose at 4°C for 1h. The preparation and characterization of antiserum to SHAP were described previously (1). Monoclonal antibody to gp85, K3 in an ascite form (2) was kindly provided by Dr. G. Tarone, University of Turin, Italy. The immunofluorescent staining of these cell layers was carried out as described previously (4). In some experiments, the staining was carried out the same way as described by Tarone et al. (2). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent immunoblotting were carried out as described previously (1).

RESULTS AND DISCUSSION

Immunofluorescence of SHAP in mouse dermal fibroblasts in the 4th day culture was located predominantly in the pericellular regions (Fig. 1A). However, treatment of the cultures with hyaluronate lyase before staining
Yoneda M et al: Serum-derived HA-binding protein, SHAP

had no obvious effect on the fluorescence pattern (data not shown). The results suggest that most, if not all, SHAP is associated with cell surface membranes. Staining of the dermal fibroblast cultures with K3 showed no immunofluorescence under any conditions used (Fig. 1B). On the other hand, K3 stained BHK cell cultures, exhibiting fluorescent localization on cell surface membranes (Fig. 1D), which was similar to that reported by Tarone et al. (2). The BHK cells were also stained with the antiserum to SHAP with a fluorescent pattern essentially similar to that of the dermal fibroblasts (Fig. 1C). SDS-PAGE of the isolated SHAP and the subsequent immunoblotting with K3 confirmed no reactivity of SHAP with K3 (Fig. 2).

If SHAP be an identical molecule to gp85, the dermal fibroblasts and the isolated SHAP should have been reactive to K3, since SHAP is derived from fetal calf serum which had also been used by Tarone et al. as a medium supplement for the culture of BHK cells. Taken together with these results, it is very likely that SHAP is a molecule different from gp85.

REFERENCES