Regulation of hyaluronan synthesis and the interaction of hyaluronan with cells

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Hyaluronan is a high molecular weight polysaccharide ubiquitously found in the extracellular matrix. It acts as a lubricant in joints and tissues and provides stability and elasticity to the extracellular matrix. In addition, hyaluronan is directly or indirectly implicated in cell growth, angiogenesis, differentiation and malignant tumor invasion (for a review see Laurent and Fraser 1992, Sherman et al. 1994). This review focuses on the regulation of the biosynthesis of hyaluronan and the formation and structure of the pericellular coat around hyaluronan producing cells.

**Regulation of biosynthesis**

Hyaluronan is synthesized by a membrane-bound enzyme and its production by human foreskin fibroblasts in culture is stimulated by growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor-\(\beta\) (TGF-\(\beta\)); the stimulatory activities do not correlate with their mitogenic activities (Heldin et al. 1989). The PDGF-induced hyaluronan synthesis is mainly mediated by the PDGF \(\beta\)-receptor. Furthermore, we have found that the signal transduction pathways through which PDGF and TGF-\(\beta\) mediate their stimulatory effects on hyaluronan synthesis involve protein kinase C and are partly dependent on protein synthesis. Direct activation of protein kinase C by phorbol 12-myristate 13-acetate also stimulates hyaluronan synthesis and this stimulation is not dependent on protein synthesis (Suzuki et al. 1995).

During tissue repair and inflammatory reactions, such as liver cirrhosis, liver regeneration after partial hepatectomy and rheumatoid arthritis, an excessive accumulation of hyaluronan is seen in tissues and serum (Engström-Laurent 1989, Vrochides et al. 1995). A possible mechanism could be that inflammatory cells produce growth factors that stimulate the synthesis of hyaluronan, other glycosaminoglycans and collagen. Recently, we showed that accumulation of hyaluronan after bleomycin-induced lung injury could in part be due to factors in bronchoalveolar lavage fluid and in conditioned medium from alveolar macrophages isolated from bleomycin-treated rats which stimulate hyaluronan biosynthesis. Part of the accumulated hyaluronan could be due to an impaired function of hyaluronan receptors on alveolar macrophages (Teder et al. 1995). Furthermore, the increased levels of hyaluronan often seen in some invasive tumors is not due to synthesis of hyaluronan by the tumor cells on their own; tumor cells secrete factors that stimulate hyaluronan synthesis by adjacent normal mesenchymal cells (Knudson et al. 1989, Asplund et al. 1993).

**The pericellular coat**

Human synovial fibroblasts as well as other cell types are surrounded in culture by hyaluronan-containing pericellular matrices, which are readily visualized by a particle exclusion assay first described by Clarris and Fraser 1968 (Fig. 1a). The size of the coat is determined by the average length of the hyaluronan molecules, and the coat seems to be attached to the cell surface in at least two different ways. One type of coat is anchored to hyaluronan receptors on the cell surface, e.g. on chondrocytes (Knudson and Knudson 1993). The other type of coat consists of newly synthesized hyaluronan which is extruded from the cell surface, e.g. of mesothelial cells (Heldin and Pertoft 1993). The function of the hyaluronan-containing coat is not well defined but it may be important to keep cells apart, to protect human synovial cells against cytotoxic lymphocytes or to contribute to the ability of glioma cells to evade cellular immune attack. Recently, we demonstrated that aggregating cartilage proteoglycan added to the culture medium of human mesothelial cells, the progenitor cells of mesothelioma, increased the compactness of the pericellular layer surrounding mesothelial cells, leading to the exclusion of bacteria with a minimum size of 0.5 \(\mu\)m (Figure 1c; Heldin et al. 1995). Furthermore, serum proteins and growth factors such as PDGF-BB and EGF are required for the formation of the coat around mesothelial cells indicating that the assembly of pericellular coats can also be regulated through extracellular signals (Heldin and Pertoft 1993).

**Discussion**

It is obvious that knowledge about the molecular
mechanisms that regulate hyaluronan synthesis and its interactions with cell surface receptors and matrix proteins are crucial for the understanding the biological function of hyaluronan.

Our studies on these processes may be of relevance for the function of hyaluronan in joint disease and the use of hyaluronan as a clinical marker. In arthritic joints both the concentration (but not the total amount), and the molecular size of hyaluronan decline in synovial fluid (Balazs et al. 1967), concomitant with local inflammation and subsequent pain and joint destruction; treatment with high molecular weight hyaluronan relieve the pain (Strachan et al. 1990). Of interest in this context is to investigate whether the composition and the functional significance of the coats around synovial cells differ between healthy individuals and patients with rheumatoid arthritis or other joint diseases.

Acknowledgements

We thank professor T.C. Laurent for support and constructive criticism of this work. This work was supported in part by grants from the Swedish Cancer Society, Gustaf V:s 80årsfond and the Medical Research Council (03X-4).

References


Localization of hyaluronan and the hyaluronan receptor ICAM-1 in rheumatoid synovia—a histochemical study

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The major site for elimination of HA from the bloodstream is via receptor mediated endocytosis by liver endothelial cells (LEC) (Fraser and Laurent 1989, Laurent and Fraser 1992). The HA receptor (HAR) on LEC has been characterized and isolated from rat LEC membranes (Forsberg and Gustafson 1991). A monospecific polyclonal antibody against the 90-100 kD receptor, which could inhibit binding of HA to LEC and LEC membranes, (termed anti-HARLEC) was raised (Forsberg and Gustafson 1991). In immunohistochemical studies the antibody stains mainly endothelium such as the sinusoids in the liver, spleen and lymph nodes, some capillaries in kidney and corneal endothelial cells (Gustafson et al. 1994, Forsberg et al. 1994). Tryptic peptides of the receptor were sequenced and found to be identical to intercellular adhesion molecule-1 (ICAM-1) (McCourt et al. 1994). ICAM-1 is normally expressed at low levels, but has been found on normal liver endothelium at the sinusoids and on the endothelium of lymph nodes, spleen and some capillaries of the kidney (Dustin et al. 1986), as well as on corneal endothelial cells (Foets et al. 1992). The localization corresponds well with anti-HARLEC staining (Gustafson et al. 1994, Forsberg et al. 1994). ICAM-1 is up-regulated in inflammation and neoplasia and is a key molecule for leukocyte adherence and transeendothelial migration (Dustin et al. 1986, Tamatani and Miyasaka 1990, Springer 1994). We have recently also shown that HARLEC/ICAM-1, expressed on tumor endothelium in mouse mastocytomas, can bind intravenously administered radiolabeled HA (Gustafson et al. 1995). The aim of this study was to investigate the relationship between HA and ICAM-1 in human rheumatoid synovia.

Materials and methods

Monoclonal antibodies: The monoclonal antibody against human ICAM-1, clone 15.2, was from Southern Biotechnology, Birmingham, U.S.A., and the monoclonal antibody towards human CD68 was from DAKO, Glostrup, Denmark.

Tissues: Rheumatic synovial membrane from patients suffering from classical seropositive rheumatoid arthritis were collected at therapeutic orthopedic synovectomies. Immunostaining: Frozen sections of 6 μm were prepared and mounted on glass slides that were coated with gelatin-kromalun. The frozen sections were fixed in cold methanol for 10 minutes, dried for 10 minutes before being washed with phosphate buffered saline (PBS). Endogenous peroxidase was blocked in 0.3% H₂O₂ in methanol, and the sections were once more washed in PBS. To block endogenous biotin and biotin-binding activity, an avidin/biotin blocking kit from Vector laboratories was used. The staining for HA was performed as described previously (Gustafson et al. 1995), using biotinylated hyaluronan binding proteins from cartilage (b-HABP). After incubation with the second antibody, or b-HABP, the sections were washed and incubated