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Soluble factors derived from human amniotic epithelial cells suppress collagen production in human hepatic stellate cells

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Abstract

Background. Intravenous infusion of human amniotic epithelial cells (hAECs) has been shown to ameliorate hepatic fibrosis in murine models. Hepatic stellate cells (HSCs) are the principal collagen-secreting cells in the liver. The aim of this study was to investigate whether factors secreted by hAECs and present in hAEC-conditioned medium (CM) have anti-fibrotic effects on activated human HSCs. *Methods.* Human AECs were isolated from the placenta and cultured. Human hepatic stellate cells were exposed to hAEC CM to determine potential anti-fibrotic effects. *Results.* HSCs treated for 48 h with hAEC CM displayed a significant reduction in the expression of the myofibroblast markers α-smooth muscle actin and platelet-derived growth factor. Expression of the pro-fibrotic cytokine transforming growth factor-β1 (TGF-β1) and intracellular collagen were reduced by 45% and 46%, respectively. Human AEC CM induced HSC apoptosis in 11.8% of treated cells and reduced HSC proliferation. Soluble human leukocyte antigen–G1, a hAEC-derived factor, significantly decreased TGF-β1 and collagen production in activated HSCs, although the effect on collagen production was less than that of hAEC CM. The reduction in collagen and TGF-B1 could not be attributed to PGE2, relaxin, IL-10, TGF-B3, FasL or TRAIL. *Conclusions.* Human AEC CM treatment suppresses markers of activation, proliferation and fibrosis in human HSCs as well as inducing apoptosis and reducing proliferation. Human AEC CM treatment may be effective in ameliorating liver fibrosis and warrants further study.

Key Words: *amnion epithelial cells, hepatic stellate cells, liver cirrhosis, placental stem cells*

Introduction

Liver fibrosis represents a wound-healing response to chronic inflammation leading to the accumulation of collagen and other extracellular matrix (ECM) proteins that can result in distortion of liver architecture and vasculature, liver dysfunction and hepatocyte dysplasia. Inflammatory liver injury can be precipitated by viral, metabolic, toxin, autoimmune and other causes. The hepatic stellate cell (HSC) is the resident liver cell at the center of collagen deposition because its response to tissue injury initiates and modulates liver fibrosis. HSC activation represents a series of cellular processes resulting in transformation to a myofibroblast-like, pro-fibrotic phenotype. The driving forces leading to this transformation are hepatocyte apoptosis (1) and resident and recruited immune cells, which are sources of cytokines such as platelet-derived growth factor-β (PDGFβ), endothelin-1, connective tissue growth factor, epidermal growth factor and transforming growth factor-β (TGF-β) (2). This pro-fibrotic response results in ECM deposition and liver fibrosis (3). If the stimulus for liver injury is removed, activated HSCs may revert to a quiescent phenotype or decrease in number through spontaneous or directed apoptosis (4). The remaining HSCs express a number of enzymes and enzyme inhibitors whose net result is fibrolysis or ECM degradation leading to regression of liver fibrosis. If liver injury is perpetuated, the ongoing fibrogenesis can lead to cirrhosis and its associated complications.

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Recently, stem cell–based therapies have been used for their liver-regenerative and anti-fibrotic properties and have been shown to be efficacious in experimental (5–7) and human liver disease (8–10). Cell sources include embryonic stem cells, induced pluripotent stem cells, and adult hematopoietic and mesenchymal stromal cells. However, there are a number of issues including availability, tumor induction (11,12) and potential ethical constraints that diminish the clinical application of some types of stem cells. One cell source that overcomes many of these limitations is the placenta, which contains several stem or stem-like cells, including amniotic epithelial cells. These cells retain stem cell characteristics and express surface markers present on embryonic and germ-line cells (13,14). Placenta-derived cells also have properties that address the safety and availability concerns with other stem cells. Human amniotic epithelial cells (hAECs) do not form teratomas *in vivo* (13,14), as has been shown with embryonic stem cells (15). Human AECs express low levels of human leukocyte antigen class IA molecules (HLA-IA) and lack class II antigens (14,16), which partially accounts for the low immunogenicity and lack of acute rejection observed when transplanted into humans (17). Human AECs are also highly abundant, and approximately 150 million cells can be isolated from one placenta (18,19). Human placentas are routinely discarded after birth, which largely obviates the ethical concerns regarding their use in human disease. On the basis of their stem cell characteristics, accessibility and safety, hAECs are a promising source for cell-based therapy.

We have shown that hAECs have an anti-fibrotic effect after xenotransplantation in murine models of liver fibrosis. In a 4-week acute model and a 12-week chronic model of carbon tetrachloride (CCl_4)-induced liver fibrosis, mice given a single intravenous dose of hAECs showed significant reduction in liver fibrosis (20,21). This anti-fibrotic effect is mediated partially through a reduction in the number of activated HSCs and through reduction in pro-fibrotic cytokine expression. The reduction in fibrosis may be through an indirect effect after hAEC modulation of pro-fibrotic macrophages or through a direct effect of hAECs on HSCs (21). This notion is suggested by the observation that infused hAECs were found in the liver in close proximity to HSCs, suggesting that hAEC soluble factors may alter HSC collagen expression. In this study, we have sought to determine whether soluble factors from hAECs inactivate HSCs and inhibit collagen expression. We also explored potential mechanisms that might explain such changes.

Methods

Human AEC isolation

Amniotic membranes were collected from healthy women with a normal singleton pregnancy undergoing cesarean section at term (37–40 weeks' gestation). Informed written consent was obtained before surgery. The study was approved by the Royal Women's Hospital Human Research Ethics Committee.

Human AECs were isolated from amnion membranes, and purity was assessed as described previously (14,19). Briefly, amniotic membranes were cut up and digested twice for 40 min at 37°C in 0.05% Trypsin: ethylenediaminetetra-acetic acid (Gibco, Grand Island, NY, USA). After washing, red blood cells were lysed by incubation in a hypotonic solution. Viable stage specific embryonic antigen-positive cells were recovered by Percoll density gradient centrifugation. Purity was assessed by means of flow cytometry for cytokeratin 7 and 8/18 (Dako, Glostrup, Denmark), and batches that were ~98% positive for the cytokeratins and had typical cobblestone morphology in culture were used. Cells were cryopreserved in 90% fetal calf serum (FCS) with 10% dimethyl sulfoxide.

Human AEC–conditioned medium

Cryopreserved hAECs from four randomly selected donor placentas were revived, pooled together and grown in Dulbecco's Modified Eagle's/Ham's F12 Medium (DMEM/F12) supplemented with 10% FCS, an antibiotic and an antimycotic, L-glutamine and 10 ng/mL epidermal growth factor (Life Technologies, Grand Island, NY, USA) on collagen IV–coated flasks (1 mg/mL; Roche, Mannheim, Germany). Flasks were kept at 37°C in a humidified 5% CO_2 atmosphere. For collection of conditioned medium (CM), confluent cells were serum-starved in DMEM/F12 and the CM was collected after 72 h. The CM was spun to remove cell debris and frozen at −80°C until needed. CM from three to five pools was used, with each pool taken from a culture of hAECs from four donor placentas. Before CM from pooled hAEC donors was used for planned experiments, individual and various combinations of pooled hAECs were assessed. Four randomly selected donors were chosen to assess for individual donor variability in regard to TGF-β1 production and Proline incorporation (collagen production); after this, 12 randomly chosen donors were cultured in three separate pools (four hAEC donors per pool) to obtain CM. Comparisons were made between individual donors and control and between individual pools and control. No significant difference was