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Follistatin allows efficient retroviral-mediated gene transfer into rat liver

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Abstract

Retroviral vectors are widely used tools for gene therapy. However, in vivo gene transfer is only effective in dividing cells, which, in liver, requires a regenerative stimulus. Follistatin is effective in promoting liver regeneration after 90% and 70% hepatectomy in rats. We studied its efficacy on liver regeneration and retroviral-mediated gene delivery in 50% hepatectomized rats. When human recombinant follistatin was infused into the portal vein immediately after 50% hepatectomy, hepatocyte proliferation was significantly higher than in control 50% hepatectomized rats. A single injection of virus particles administered 23 h after follistatin infusion resulted in more than 20% gene transduction efficiency in hepatocytes compared to 3% in control rats. It is concluded that a single injection of follistatin induces onset of proliferation in 50% hepatectomized rats and allows efficient retroviral-mediated gene transfer to the liver.

Keywords: Follistatin; Hepatectomy; Liver regeneration; Retroviruses; In vivo gene transfer

Hepatic gene therapy is an attractive approach to the treatment of metabolic defects or serum protein deficiencies. However, treating genetic diseases by therapeutic gene delivery poses a number of problems, including the need for a stable, therapeutic expression of the transferred genes. Gene delivery using retroviruses results in long term expression because they integrate into cell chromosomes. However, the use of retroviruses for in vivo gene transfer has been hampered by the need for cell proliferation since retroviruses only integrate into dividing cells [1]. In adult livers, hepatocytes are quiescent cells, so that all in vivo delivery attempted to date has required a 70% partial hepatectomy [2,3] or the administration of a liver toxin [4] prior to injection of the retrovirus into the portal vein. However, excessive surgical removal of liver tissue or chemical chronic hepatitis can lead to liver failure. Lentiviral vectors have been used recently for gene transfer into the liver, but the ability of these vectors to efficiently transduce

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quiescent hepatocytes remains controversial, and lentivirus-mediated gene transfer is greatly enhanced when delivered during hepatocyte proliferation [5]. Also, the use of the VSV-G envelope protein allows a number of non-parenchymal cells to be transduced [6].

Recently, dogs with mucopolysaccharidosis VII were injected intravenously at 2–3 days of age with a retroviral vector expressing canine β-glucuronidase and this treatment prevented the clinical manifestations of a lysosomal storage disease in the animals [7]. However, targeting of highly dividing hepatic stem cells in newborn remains a potential risk [8]. Hepatocyte growth factor (HGF) plays a primary role in liver regeneration [9,10]. HGF has been used to induce proliferation of rat liver and several injections of HGF lead to an average of 1.3% of transduced hepatocytes [11]. Transduction efficiency increased to 6.9% when a combination of elevated doses of tri-iodothyronine (T3) and rHGF was infused in rat portal vein [12]. However, since the receptor to HGF, c-met, is a protooncogene the expression of which is found elevated in numerous tumours, stimulation of c-met tyrosine kinase through an autocrine mechanism needs to be carefully documented. Another combination of pharmaceutical compounds, cyproterone acetate and T3, resulted in the transduction of 5% hepatocytes after two virus infusions [13]. However, the efficacy may be insufficient for the treatment of a number of metabolic diseases.

Follistatin is a protein that binds activin A, a member of the TGF-β superfamily. Human follistatin has been shown to block the inhibition of DNA synthesis by activin A and to enhance epidermal-growth factor induced DNA synthesis in cultured rat primary hepatocytes [14,15]. In addition, it has been demonstrated that endogenous activin negatively regulated hepatocyte proliferation using antisense oligonucleotides directed against activin in vitro [16]. Intraportal administration of a single dose of follistatin accelerated liver regeneration in adult rats after 70% or 90% hepatectomy and was more efficient at promoting liver regeneration than HGF [17–19]. This growth effect of follistatin was augmented by its repeated administration. It was also reported that administration of follistatin to normal young rats induced DNA synthesis and liver enlargement [20].

These observations suggested that activin plays a critical role in maintaining optimal liver size and that follistatin effects on hepatocyte proliferation were a consequence of its ability to block the action of endogenous activin.

In the present study, we have addressed the issue of whether follistatin could be used to augment liver regeneration after a limited extent of hepatectomy and to allow efficient gene delivery to the liver using retroviral vectors.

We report that intraportal injection of follistatin to 50% hepatectomized rats enhances liver regeneration compared to control hepatectomized rats and that the mitogenic effect of follistatin made it possible to achieve efficient hepatocyte transduction using recombinant retroviral vectors.

Materials and methods

Animals. The rats used were young Wistar males weighing 150–180 g. All animals were maintained on standard laboratory chow and food. Water was provided ad libitum. The animals were treated according to the European Community laws for animal care.

Preparation of virus stocks and virus concentration. The amphotropic recombinant retroviruses carrying the β-galactosidase gene were produced by the FLY TA7 cell line, which has been previously described [21]. The cells were grown at 37 °C in the presence of 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated new-born calf serum, 2 mM glutamine, and antibiotics (penicillin, streptomycin) (Eurobio, Les Ulis, France). Twenty hours before viral harvest, 5 ml of fresh medium containing 2% fetal calf serum was added to the culture dishes. For each experiment, 150–300 ml of viral supernatant was collected, filtered through a 45 μm filter (Corning Incorporated, New York, USA), and immediately concentrated by tangential flow using a cartridge containing cylindrical microfibres of 40 kDa (Filtral Hospal, Gambro Group, France). The concentrated samples were immediately frozen and stored at −80 °C. Virus titres were determined in duplicate prior to and after concentration by infection of NIH3T3 cells. The cells were stained 48 h later with 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-gal) as previously described [22] and the titre was defined by the number of β-gal-positive foci multiplied by the dilution factor and expressed as "blue colony forming unit"/ml (bcfu/ml).

Surgical procedures. The rats were anesthetized with 10 mg/100 g ketamine delivered intraperitoneally. Seventy percent hepatectomy involved the removal of the median and left lateral lobes [23]. Fifty percent hepatectomy involved the removal of the left lateral lobe and the left part of the median lobe. Rats were closed by two-layer running sutures.

Saline (1 ml) containing or not 1 μg follistatin was injected into the portal vein at the time of hepatectomy.

BrdU injection and immunochemical staining. BrdU (Sigma Chemical, St. Louis, USA) (30 mg/rat, diluted in saline) was injected intraperitoneally into rats at 18, 20, 24, 28 or 33 h after hepatectomy (four animals per time point). The rats were killed 2 h later and liver fragments were fixed in 10% buffered formalin. The liver tissues were embedded in paraffin and 5-μm sections were cut. The sections were pre-incubated with normal goat serum (1:50 in PBS) (Dako, Glostrup, Denmark) and then with a mouse monoclonal antibody against BrdU (1:20, Dako) for 1 h at room temperature. Slides were rinsed with PBS and goat anti-mouse IgG (1:100, Dako) was applied for 30 min. After rinsing, the slides were incubated with the avidin–biotin complex (1:100, Vectastain ABC kit, Vector) for 1 h. The sections were counterstained with haematoxylin–eosin. The BrdU labelling index (LI) was determined as the average number of BrdU-positive nuclei per 1000 random parenchymal cells in 10 fields around portal spaces and 10 fields around centrolobular veins under a light microscope (Olympus-BH2). As a negative control, the primary antibody was replaced by PBS.

Each time point represents the sum of cycling hepatocytes during the 2-h period between BrdU injection and biopsy/sacrifice.

Hepatocyte transduction and detection of transduced cells. One to three millilitres of virus supernatant, containing 8 μg/ml polybrene