Domestic Animal Endocrinology 62 (2018) 39–48

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Species-specific control of hepatocyte growth factor expression and production in adipocytes in a differentiation-dependent manner

D. Yamaji, M.M. Soliman, A. Kamikawa, T. Ito, M.M. Ahmed, Y. Okamatsu-Ogura, M. Saito, K. Kimura*

Laboratory of Biochemistry, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

ARTICLE INFO

Article history:
Received 27 June 2017
Received in revised form 22 August 2017
Accepted 6 September 2017

Keywords:
Adipocyte
Fibroblast
Hepatocyte growth factor
NK4
Mammary gland

ABSTRACT

Hepatocyte growth factor (HGF) is a mesenchymal cell-derived factor that regulates cell growth, cell motility, and morphogenesis. Since there are conflicting reports on HGF-producing cells, we herein examined HGF activity in conditioned medium (CM) of bovine and mouse preadipocytes before and after adipogenic differentiation. CM of bovine adipocytes and mouse preadipocytes induced the morphogenesis of mammary epithelial cells that was inhibited by an NK4 HGF antagonist, whereas CM of bovine preadipocytes and mouse adipocytes did not. *HGF* mRNA expression was increased by a treatment with dexamethasone and isobutylmethylxanthine in bovine as well as human cells, whereas it was decreased in rodent cells. It was unfortunate that *HGF* gene promoter activity failed to reflect *HGF* mRNA expression in these cells. After actinomycin D treatment, expression of *HGF* mRNA remained stable in pre- and differentiated bovine adipocytes and mouse pre-adipocytes, whereas rapidly decreased in mouse-differentiated adipocytes. These results indicate that expression and production of HGF are regulated in a species-specific adipogenic differentiation-dependent manner and suggest that the decrease in *HGF* mRNA in mouse differentiated adipocytes is, at least in part, mediated by differentiation-dependent loss of its stability.

1. Introduction

Hepatocyte growth factor (HGF), also known as a scatter factor, is a heparin-binding glycoprotein mainly derived from the stroma. HGF acts on a number of epithelial cells and endothelial cells expressing the c-Met proto-oncogene, a HGF receptor, to induce cell proliferation, motility, differentiation, and morphogenesis [1,2]. Mouse mammary fat pad transplantation experiments showed that stromal cells, in response to estrogen, produce HGF to induce epithelial branching through the activation of the epithelial c-Met receptor [3,4]. Thus, HGF/c-Met pathway is involved in the embryonic and postnatal development of the mammary gland [1,3–8].

The importance of these stromal-epithelial interactions during mammary gland development has been confirmed by analyses of genetic fat-ablated mice, showing that adipocytes are required for proper mammary gland development during puberty and for maintaining the ductal architecture in the adult mammary gland [9,10]. However, it is interesting to note that the mouse stromal microenvironment is able to support mouse mammary epithelial growth, but not human epithelial growth in vivo [11,12], and also that the humanization of the mouse stroma with

* Corresponding author. Tel./fax: +81-11-757-0703.
E-mail address: k-kimura@vetmed.hokudai.ac.jp (K. Kimura).

0739-7240/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.domaniend.2017.09.001

an immortalized mammary fibroblast injection enables transplanted human mammary organoids to develop in mice [13], suggesting a species difference in the stromal microenvironment. Moreover, significant histological differences have been identified between the adipose-rich rodent mammary fat pad and that of the fibrous human breast stroma or mammary stroma in livestock species [3,14–17].

Heterodimeric biologically active HGF consisting of two polypeptides, α-chain (62 kDa) and β-chain (32–34 kDa), is detected in the conditioned medium (CM) of murine mammary fibroblasts [4,6] and human and mouse fibroblastic cell lines including 3T3-L1 preadipocytes [5,6,18–20], although a single-chain inactive HGF precursor (85 ∼ 95 KDa) is mainly produced by differentiated 3T3-L1 adipocytes [20,21]. However, the CM of human mammary fibroblasts does not exhibit HGF activity, while the cells express its mRNA [6], whereas secretion of the HGF protein is greater by freshly isolated human adipocytes than by stromal cells [22]. Moreover, serum HGF levels were reported to be 3-fold higher in obese human subjects than in lean subjects [22], suggesting a positive relationship between HGF secretion and fat content in humans. Therefore, species-specific differences may exist in the expression and production of HGF between fibroblasts and adipocytes.

In the present study, to clarify the species-specific control of HGF production, we compared the production of HGF by bovine preadipocytes and mouse 3T3-L1 cells before and after their adipogenic differentiation.

2. Materials and methods

2.1. Adipocyte culture and preparation of CM

Experimental procedures and care of animals were conducted in accordance with the Guidelines of the Animal Care and Use of Hokkaido University, Japan, and the protocol was approved by the Committee for the Care and Use of Laboratory Animals in Hokkaido University.

Bovine stromal vascular cells (preadipocytes) from subcutaneous adipose tissue were prepared as described previously [23]. In brief, the tissues obtained from three nonpregnant, nonlactating Holstein cows (2–3 years old) were digested with collagenase solution (2 mg/mL) at 37°C for 90 min. After filtered through nylon mesh with 80 μm pore, the cells mainly consisting of stromal vascular cells were collected by centrifugation and treated with an erythrocyte lysis buffer (154-mM NH_4Cl, 10-mM $KHCO_3$, and 1-mM EDTA) for 5 min. The cells were further washed twice with Dulbecco's modified eagle medium (DMEM) containing 10% fetal calf serum (FCS), 100-U/mL penicillin, and 100-μg/mL streptomycin were cultured on collagen-coated dishes. The cells between third and fifth passages were used for the experiments.

Human subcutaneous preadipocytes were purchased from Cambrex (Walkersville, MD). Immortalized mouse preadipocytes, termed HB2 cells, derived from the brown adipose tissue of p53-deficient mice were established as described previously [24]. In a similar way to the bovine cells, rat subcutaneous preadipocytes from the inguinal white adipose tissue of newborn Wistar rats (Japan SLC Inc, Shizuoka, Japan) and immortalized preadipocytes, termed HW cells, from the inguinal white adipose tissue of p53-deficient mice were prepared.

The preadipocytes of four species and mouse 3T3-L1 preadipocytes were cultured in either DMEM or DMEM/F-12 supplemented with 10% and 1.5% FCS. After reaching confluency, cells were treated with isobutylmethylxanthine (IBMX, 0.5 mM, Sigma) and dexamethazone (Dex, 1 μM, Sigma) for 2 d to induce differentiation to mature adipocytes, followed by a treatment with insulin (10 μg/mL), troglitazone (Tro, 10 μM, Sigma), and/or T_3 (50 nM, Sigma).

Confluent preadipocytes or differentiated adipocytes 4 or 13 d after the addition of IBMX and Dex were rinsed twice and cultured for a further 48 h with basal medium for the bovine mammary epithelial cells (BMEC) culture as described below. The culture medium was then collected, centrifuged at 1,500 rpm for 5 min to remove cell debris, and the supernatant was passed through a filter (0.22 μm in pore size, Advantec Toyo, Tokyo, Japan) and kept as CM at −30°C for up to 3 mo.

2.2. Isolation and three-dimensional culture of BMECs

BMEC were prepared from the mammary gland tissue of a 28-mo-old lactating cow (247 d of pregnancy) by Percoll (Amersham Pharmacia Biotech, Piscataway, NJ) density gradient centrifugation, following a treatment with tissue-digestive enzymes as described previously [25,26]. The purity of isolated cells was assessed by detecting cytokeratin, a marker of the epithelium, and vimentin, a marker of stromal cells. More than 95% of purified cells were stained with the anti-cytokeratin antibody, but not with the anti-vimentin antibody (Supplementary Fig. S1). BMEC were stored in liquid nitrogen until used.

BMEC (5×10^4 cells) were embedded in 0.15% collagen gels (Cellmatrix type I-A, Nitta Gelatin, Tokyo, Japan) using 24-well plates (BD Biosciences, San Jose, CA) according to the manufacturer's instructions and cultured with basal medium [DMEM/F-12 (Sigma-Aldrich, St. Louis, MO), bovine serum albumin (2 mg/mL, Sigma), penicillin (100 units/mL), streptomycin (100 μg/mL), gentamicin (10 μg/mL, Invitrogen, Carlsbad, CA), amphotericin B (250 ng/mL, Fungizone, Invitrogen)] supplemented with holo-transferrin (10 μg/mL, Invitrogen), insulin (10 μg/mL, Invitrogen), cortisol (5 μg/mL, Invitrogen), epidermal growth factor (10 ng/mL, Wako Pure Chemical, Osaka, Japan), and cholera toxin (10 ng/mL, Wako). One wk after the onset of the culture, collagen gel was digested with M199 medium (Sigma) supplemented with 1% collagenase (Wako) and 0.1% soybean trypsin inhibitor, and then with trypsin-EDTA (Invitrogen) to recover BMEC.

Cells were cultured again in collagen gel for the indicated periods with the CM of pre- or differentiated adipocytes as described above or basal medium containing recombinant human HGF (100 ng/mL, Mitsubishi Pharma Co, Tokyo, Japan) as an overlaid solution. The overlaid solution was changed every 2 d. In some experiments, NK4, an HGF antagonist (200 ng/mL), a gift from Drs Mizuno and Nakamura [27,28], was added to CM.