EXPERIMENTAL CELL RESEARCH 312 (2006) 1856–1864

available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/yexcr

Research Article

The use of small interfering RNAs to inhibit adipocyte differentiation in human preadipocytes and fetal-femur-derived mesenchymal cells

Y. Xu[a], S.-H. Mirmalek-Sani[a], X. Yang[a], J. Zhang[b], R.O.C. Oreffo[a,*]

[a]Bone and Joint Research Group, Developmental Origins of Health and Disease Division, University of Southampton, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, UK
[b]Division of Clinical Sciences, University of Warwick, CSB, Walsgrave Hospital Campus, Coventry CV2 2DX, UK

ARTICLE INFORMATION

Article Chronology:
Received 6 January 2006
Revised version received
13 February 2006
Accepted 15 February 2006
Available online 17 April 2006

Keywords:
Small interfering RNA
Peroxisome proliferator activated receptor gamma
Human preadipocytes
Human mesenchymal cells
Adipocyte differentiation
Differentiation

ABSTRACT

RNA interference (RNAi) has been used in functional genomics and offers innovative approaches in the development of novel therapeutics. Human mesenchymal stem cells offer a unique cell source for tissue engineering/regeneration strategies. The current study examined the potential of small interfering RNAs (siRNA) against human peroxisome proliferator activated receptor gamma (PPARγ) to suppress adipocyte differentiation (adipogenesis) in human preadipocytes and fetal-femur-derived mesenchymal cells. Adipogenesis was investigated using cellular and biochemical analysis. Transient transfection with PPARγ-siRNA using a liposomal-based strategy resulted in a significant inhibition of adipogenesis in human preadipocytes and fetal-femur-derived mesenchymal cells, compared to controls (cell, liposomal and negative siRNA). The inhibitory effect of PPARγ-siRNA was supported by testing human PPARγ mRNA and adipogenic associated genes using reverse transcription polymerase chain reaction (RT-PCR) to adiponectin receptor 1 and 2 as well as examination of fatty acid binding protein 3 ($FABP_3$) expression, an adipocyte-specific marker. The current studies indicate that PPARγ-siRNA is a useful tool to study adipogenesis in human cells, with potential applications both therapeutic and in the elucidation of mesenchymal cell differentiation in the modulation of cell differentiation in human mesenchymal cells.

Introduction

RNA interference (RNAi) is an evolutionary conserved post-transcriptional mechanism of gene silencing induced by sequence-specific double-stranded RNA [1]. Duplex RNA of 21–23 nucleotides, termed small interfering RNA, or siRNA, triggers RNAi processing resulting in specific endonucleolytic cleavage of mRNA [2–6]. It is believed that siRNAs guide a nuclease-containing protein complex, referred to as RNA-induced silencing complex, to its cognate mRNA resulting in degradation of the substrates [2,7,8]. Over the last few years, RNAi has been used in functionally analyzing the genome of *C. elegans* [9,10], identifying genes contributing to genome stability and a core set of fat regulatory genes as well as

* *Corresponding author*. Fax: +44 23 8079 6141.
E-mail address: roco@soton.ac.uk (R.O.C. Oreffo).

0014-4827/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2006.02.016

pathway-specific fat regulators. Thus, RNAi has emerged as a potential new modality in the development of novel therapeutic approaches for certain human diseases [11–15].

In the adult, the cell responsible for bone formation, the osteoblast, is derived from a multipotent marrow stromal fibroblastic stem cell termed the mesenchymal stem cell. These mesenchymal stem cells can give rise to cells of the adipogenic, reticular, osteoblastic, myoblastic and fibroblastic lineages and generate progenitors committed to one or more cell lines with an apparent degree of plasticity or interconversion [16–19]. Mesenchymal cells offer tremendous potential as vehicles for genes in gene therapy protocols and in the generation of transplantation tissues and organs in tissue regeneration. It has long been recognized that an association exists between an increase in marrow adipose tissue and osteopenia with increasing age and in a variety of experimental and pathogenic conditions, such as disuse osteoporosis and glucocorticoid-induced osteoporosis [20,21]. Recent in vitro studies have indicated some of the molecular mechanisms which may be involved in determining the lineage of stromal progenitors toward osteogenic or adipogenic cells that underlie these clinical findings. Gap-junction communication [22] and mitogen-activated protein kinase [23] have been shown to be involved in the regulation of stromal progenitors or human mesenchymal stem cells differentiation along the osteogenic or adipogenic lineages. Kha et al. [24] found that specific oxysterols could regulate lineage-specific differentiation of mesenchymal stem cells into osteogenic cells while inhibiting differentiation into adipogenic cells, adding further support to the plasticity or interconversion potential that may exist between the lineages and that the adipocytic and osteogenic cells share a common lineage. Thus, inhibition of adipogenesis may provide an approach to prevent or treat osteoporosis or other bone diseases and has been advocated by a number of groups (reviewed extensively by Gimble, Nuttall and other authors [25–27]. In addition, given the potential for interconversion [28] and shared lineage, intense interest surrounds the use of human adipose tissue as a source of osteogenic cells for tissue engineering [26–29].

Recently, Hoelters et al. reported knock-down of an exogenous gene, enhanced green fluorescent protein (EGFP), in human mesenchymal cells with transfection of specific siRNA using a liposome-based strategy [30]. However, to date, functional consequences of such transfection strategies have not been reported in human mesenchymal cells. Peroxisome proliferator activated receptor gamma [31,32], or PPARγ, is a nuclear receptor and regarded as a master regulator of adipogenesis and metabolic homeostasis [33]. The critical role of PPARγ in adipogenesis and insulin sensitization has been demonstrated in PPARγ knockout animals [34–36], and a current study suppressing adipogenesis in a murine preadipocyte cell line (3T3-L1) using an adenovirus-vector-mediated RNAi system specific to PPARγ has been reported [37]. Suppressing PPARγ function through the TAK_1/TAB_1/NIK cascade leads to cellular differentiation towards osteoblasts rather than adipocytes in multipotent mesenchymal stem cells [38], suggesting that PPARγ acts as a gatekeeper of multipotency in mesenchymal cells. Human PPARγ was a target gene in this present study to inhibit adipogenesis in human preadipocytes and fetal-femur-derived mesenchymal populations. Given the emerging potential to target PPARγ and the challenges of an increasing ageing population, inhibition of bone marrow adipogenesis may provide an alternative therapeutic approach to either prevent or treat osteopenic disorders [27]. This study set out to examine whether adipogenesis could be inhibited in human preadipocyte cells and, critically, in human fetal-femur-derived mesenchymal populations. The ability to modulate adipocyte differentiation and lineage fate has tremendous therapeutic implications for an increasing ageing population should facile strategies be developed to manipulate cell differentiation.

Methods and materials

Materials

Minimum Essential Medium Alpha Medium (α-MEM) was purchased from GIBCO (UK). Fetal Calf Serum (FCS), growth factors including Insulin Transferrin Selenium Solution (ITS, I3146), staining solutions and all other biochemical reagents were obtained from Sigma-Aldrich (UK) unless otherwise stated. Reagents used in cDNA synthesis were purchased from Promega (UK). Opti-MEM I Reduced Serum Medium, Lipofectamine 2000 and BLOCK-iT™ Fluorescent Oligo were purchased from Invitrogen Life Technologies (UK). Anti-human FABP-3 polyclonal antibody (AF1678) was from R&D Systems (UK).

Human preadipocytes

Cryopreserved human subcutaneous preadipocytes were purchased from Zen-Bio, Inc. (USA). Cells were grown in preadipocyte medium (PM-1; DMEM/Ham's F-12 medium (1:1, v/v), HEPES pH 7.4, fetal bovine serum, penicillin, streptomycin, amphotericin B) in 75 cm^2 flask at 37°C in a humidified atmosphere of 5% CO_2. Prior to confluence, cells were trypsinized and plated onto 6-well plates. Cells were maintained in PM-1 before and after siRNA transfection until cells reached confluence whereupon adipogenesis was induced.

Human fetal-femur-derived mesenchymal cells

Human fetal femurs were obtained following termination of pregnancy according to guidelines issued by the Polkinghome Report and with ethical approval from the Southampton and South West Hampshire Local Research Ethics Committee. The femurs were placed in sterile phosphate-buffered saline (PBS) and surrounded skeletal muscle removed. Femurs were dissected into pieces (femur explants) and incubated in α-MEM containing 10% FCS and 100 units/ml penicillin and 100 μg/ml streptomycin (basic cell culture medium) at 37°C in a humidified atmosphere of 5% CO_2. Culture medium was changed weekly. Explant cultures were trypsinized at 80% confluence and plated onto 6-well plates for siRNA transfection/adipogenic induction. Cells were used for siRNA transfection/adipogenic induction at passage 2 or 3 only. Fetal-femur-derived mesenchymal populations have previously been shown to differentiate into adipogenic, osteogenic or