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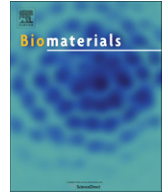

The prolonged survival of fibroblasts with forced lipid catabolism in visceral fat following encapsulation in alginate-poly-L-lysine

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ABSTRACT

Although alginate-poly-L-lysine (AP_L) encapsulation of cells producing bioactive peptides has been widely tested, it is unknown whether AP_L supports lasting catabolic functions of encapsulated cells in adipose tissue, which are required for obesity reduction. We tested functions of AP_L-encapsulated fibroblasts isolated from wild-type (WT) and aldehyde dehydrogenase 1a1 knockout mice (KO), which resist obesity on a high-fat (HF) diet, have a higher metabolic rate, and express increased levels of thermogenic uncoupling protein-1 (Ucp1) in their deleterious visceral fat depots compared to WT mice. To enable in vivo detection and quantification, fibroblasts were stably transfected with green-fluorescent protein. WT- or KO-containing microcapsules were injected into two visceral depots of WT mice fed an HF diet. Eighty days after transplantation, microcapsules were located in vivo using magnetic resonance imaging. KO microcapsules prevented weight gain in obese WT mice compared to a mock- and WT capsule-injected groups on an HF diet. The weight loss in KO-treated mice corresponded to lipid reduction and induction of thermogenesis in the injected visceral fat. The non-treated subcutaneous fat was not altered. Our data suggest that the AP_L polymer supports long-term catabolic functions of genetically-modified fibroblasts, which can be potentially used for depot-specific obesity treatment.

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1. Introduction

Microcapsules fabricated from alginate-poly-L-lysine (AP_L) were established as delivery vehicles for engineered cells producing bioactive peptides, such as insulin, leptin, and growth factors [1–4]. In contrast, the ability for AP_L microcapsules to support the long-term survival and functions of cells catabolizing metabolites has not been examined. Application of encapsulated catabolically-active cells will require different strategies for microcapsule tracking, monitoring transplanted cell viability, functions, and interaction with the host cells. Metabolic disorders, especially obesity, can benefit from the engraftment of cells with forced lipid utilization, energy dissipation, and/or high metabolic rate.

Obesity in humans and rodents is a multifactorial disorder depending on genetic factors, diet, energy expenditure, and complex interaction between different organs [5,6]. Adipose tissue is comprised of two major types lipid-storing white fat depots (e.g. visceral and subcutaneous (inguinal depots) and brown fat, which dissipates energy in the form of heat (thermogenesis) [7,8]. Thermogenic adipocytes (thermocytes) of different embryonic origins reside in different locations: brown adipose tissue or interspersed in white adipose tissue [9,10]. These thermocytes are called ‘brown in white’, ‘brite’, ‘beige’, or multilocular cells [11]. Independent of their origin, thermocytes express uncoupling protein 1 (Ucp1), which can increase daily energy expenditure by up to 20% in humans [12].

Visceral fat is a fundamental and integral component of obesity. Visceral fat secretes cytokines that provoke insulin resistance and

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chronic inflammation [13,14], which double the risk of cancer, cardiovascular diseases, and premature death from all causes [15–20]. In contrast, the progressive loss of subcutaneous tissue noted with aging correlates with development of insulin resistance, and type 2 diabetes [14]. Current pharmacologic or dietary therapies aimed at the reduction of obesity do not discriminate between subcutaneous and visceral adipose depots and are ineffective, especially in aging and disabled patients. Another critical shortcoming of these approaches is the poor compliance of patients to these regimens with inevitable attrition of therapeutic efficacy even after invasive bariatric and liposuction/lipectomy [21,22]. In the absence of effective treatment, obesity rates are increasing in epidemic proportions worldwide with 65 million more obese adults expected in the US alone by the year 2030 [23].

In mouse models, several genes playing a critical role in development of obesity have been identified [2,6]. Traditionally appetite- and energy expenditure- regulating hormones like leptin were considered for obesity treatment [2], but failed due to the development of leptin resistant conditions in rodents [24] and humans [25] with general obesity. Microcapsules with gut K cells secreting leptin also showed promising results in leptin deficient ob/ob mice; however, the microcapsules lack a therapeutic effect on high-fat (HF) diet-induced obesity in wild-type C57Bl/6J (WT) [2]. Many genes regulating thermogenesis or thermocyte differentiation successfully offset obesity on regular diets consistent with their role in energy expenditure [9,26,27] in genetically-modified animals. These genetic modifications were less effective on animals fed obesogenic diets (reviewed in [5]). Effects of engrafted thermocytes or cells catabolizing lipids for studies on energy balance in obese mice on HF diets have not been reported. It is unclear whether encapsulation is a suitable technology for the transplantation of these cells.

In our previous studies, we found remarkable resistance to HF diet-induced visceral obesity in aldehyde dehydrogenase 1a1 deficient (*Aldh1a1*$^{-/-}$) mice [28]. In these mice, the altered balance in bioactive vitamin A metabolites production increases metabolic rate, expression of thermogenic genes, and body temperature [28]. Our study examined how AP_L material supports survival and function of *Aldh1a1*$^{-/-}$ fibroblasts engrafted into visceral fat.

2. Materials and methods

2.1. Chemicals and reagents

We purchased reagents from Sigma–Aldrich (St. Louis, MO), cell culture media from Invitrogen (Carlsbad, CA), antibodies from Cell Signaling Technology (Danvers, MA) for Gapdh, Atgl, Ucp1, and β-actin; from Abcam (Cambridge, MA) for GFP (monoclonal), and β-tubulin; from LI-COR Biosciences (Lincoln, Nebraska) for secondary antibodies. FITC-insulin and Alexa Fluor 488-labeled IgG were from Invitrogen.

2.2. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee.

Aldh1a1$^{-/-}$ mice were provided by Dr. Duester (Sanford-Burnham Medical Research Institute). Their construction and characterization was described before [29]. The mechanisms responsible for resistance of *Aldh1a1*$^{-/-}$ mice to visceral obesity and an HF diet-induced obesity were reported previously in [28,30].

2.3. Animal studies and metabolic measurements

Study 1. Four (4 weeks old) C57BL/6J (WT) and six *Aldh1a1*$^{-/-}$ female mice were fed a high-fat diet (HF, 45% kcal from fat with standard vitamin A content, 4 IU/g, D12451, Research Diets Inc., New Brunswick, NJ) for 14 months. Visceral (periovarian) subcutaneous (inguinal), and brown fat were collected at the end of the study for mRNA analysis and embedded into paraffin for histological examination (see 2.9).

Encapsulation study 2. Fifteen 3-month-old WT female mice were fed a high-fat diet for 90 days. Then mice were randomly assigned into three groups ($n = 5$ each):

1) injected with vehicle (1 mL sterile phosphate buffer (PBS),
2) injected with encapsulated ^{G}WT fibroblasts (0.5*10^{6} cells in 1 mL PBS per visceral depot)
3) injected with encapsulated ^{G}KO fibroblasts (0.5*10^{6} cells in 1 mL PBS per visceral depot).

Mice were injected with vehicle or encapsulated cells into both visceral (periovarian) depots and maintained on a similar HF diet for 80d. Two weeks prior to the end of the study, metabolic parameters in the treated mice were measured by indirect calorimetry (CLAMS, Columbus Instruments, Columbus, OH) at ambient temperature (22 °C) with 12 h light/dark cycles. Animals were fed the same HF diet and water provided *ad libitum*. Mice were placed individually and allowed to acclimatize in the chambers for 12 h, and O_2 consumption, CO_2 production, energy expenditure, and locomotor activity were measured for 24 h. Based on these data, respiratory quotient or exchange ratio (V_{CO2}/V_{O2}) and Δ heat values were calculated by CLAMS. Metabolic rate (MR) was calculated based on Romijn and Lokhorst formula as described previously [31]: $MR = 16.18*V_{O2}+5.02*V_{CO2}$. For glucose tolerance tests (GTT) mice were fasted overnight and were injected with a single intraperitoneal 25% glucose dose (0.004 mL/g body weight). Blood glucose was measured by One Touch Ultra Glucometer (LifeScan).

2.4. Magnetic resonance imaging (MRI)

At the end of the study 2, body fat was measured by 9.4T Bruker BioSpin widebore scanner as described before [30]. Multi Slice Multi Echo sequence (repetition time 1400 ms; echo time 12 ms; flip angle 180°; matrix 128 × 128; 4 averages) was used to acquire a total of 80 1-mm-thick images per mouse scanning from the tail to the head. ParaVision 4.0 software was used for MR acquisition and reconstruction. All image processing and analysis was performed in OsiriX software (The Osirix Foundation, Geneva, Switzerland).

2.5. Organ collection, lipid extraction, and western blot analysis

After MRI mice were anesthetized, blood was collected by cardiac puncture. EDTA plasma was prepared by centrifugation. Plasma was analyzed for non-esterified fatty acids (NEFA) using colorimetric assay (Wako Diagnostics, Richmond, USA). One whole subcutaneous and visceral (peri-ovarian) fat pads were dissected and homogenized in RIPA buffer with protease inhibitors. Aliquots from fat pad homogenates were used for lipid extraction, protein (Bicinchoninic Acid Kit) measurements, and western blots. Lipids (total lipophilic compounds) were extracted from 100 μL RIPA buffer using first, 10 μL ethanol, and then 700 μL hexane and 50 μL chloroform. After 1 min vortexing and centrifugation (4000 rpm), the organic phase was collected and dried under N_2. For Western blot analyses, tissue lysates were separated on 10% acrylamide gel under reducing conditions. After transfer to a polyvinylidene fluoride membrane (Immobilon-P, Millipore), proteins were analyzed using an Odyssey Infrared Imaging System (LI-COR). Images were quantified by ImageJ software.

2.6. Immunohistochemistry

The remaining fat pads were embedded in paraffin and in OCT for immunohistochemical analysis and laser microdissection. Sections (14 μm) were stained with hematoxylin and eosin (H&E) using a modified hematoxylin QS procedure [32] followed by dehydration in graded alcohol [33] or with Ucp1 polyclonal rabbit antibodies (both 1:1000 dilution).

2.7. Encapsulation

The phase microencapsulation technique for preparation of alginate-poly-L-lysine (AP_L) microcapsules has been performed as described [34]. The cell suspension (2 × 10^{6} cells/ml) were suspended in 2% sodium alginate solution (Sigma, St. Louis, MO, MW 12,000–80,000 g/mol, 100–300 cps Brookfield viscosity). Then, this suspension was extruded through a inner diameter of 0.337-mm needle (23 gauge) into a 100 mM $CaCl_2$ solution, using Encapsulation Unit (Nisco Engineering AG, Switzerland) at 5.4 kV to form calcium alginate gel beads. Nozzle size, flow rate, and vibration frequency were identical for all encapsulation experiments. The gel beads trapped with cells were solidified in 100 mM $CaCl_2$ for 20 min, and then incubated with 0.05% (w/v) poly-L-lysine (MW 20,700, Sigma, St. Louis, MO) to form alginate-poly-L-lysine membrane around the surface. The membrane-enclosed gel beads were further suspended in 50 mM sodium citrate to liquefy the alginate core. Encapsulated fibroblasts were cultured with floating status in standard medium instead of embedding at the sodium alginate gel within the microcapsule and were maintained in culture under standard conditions for up to 30d.

2.8. Cell line engineering

Embryos (16 days-old) were collected from C56/BL6 (WT) and *Aldh1a1*$^{-/-}$ mice. WT and *Aldh1a1*$^{-/-}$ fibroblast cell lines were derived from embryonic fibroblasts and immortalized by following a classical protocol by Green & Meuth [35]. Then, WT and *Aldh1a1*$^{-/-}$ fibroblasts were transfected with PReceiver-Lv08GFP (Lentigen)

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