Stem Cell Research 33 (2018) 25–35

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

High fat diet activates adult mouse lung stem cells and accelerates several aging-induced effects☆

Ahmed E. Hegab[a,*,1], Mari Ozaki[a,1], Fatma Y. Meligy[a,b], Shizuko Kagawa[a], Makoto Ishii[a], Tomoko Betsuyaku[a]

[a] Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Tokyo, Japan
[b] Department of Histology, Faculty of Medicine, Assiut University, Assiut, Egypt

ARTICLE INFO

Keywords:
High fat diet
Aging
Alveolar cells
Lung stem cells
Mitochondria
Calorie restriction

ABSTRACT

High fat diet (HFD) decreases the lifespan of mice, and is a risk factor for several human diseases. Here, we investigated the effects of a HFD on lung epithelial and stem cells and its interaction with aging. Young and old mice were fed with either a standard diet (SD) or a HFD then their trachea and lung were examined for histological changes, inflammation, and mitochondrial function. Their stem cell function was examined using the *in vitro* organoid/colony forming efficiency (CFE) assay. Aging reduced the number of tracheal basal and alveolar type-2 (AT2) cells. HFD significantly increased the number of AT2 cells. Aging also caused a significant increase in lung inflammation, and HFD caused a similar increase, in young mice. Aging reduced mitochondrial mass and function, and increased reactive oxygen species. In young mice, HFD caused mitochondrial changes similar to the aging-induced changes. Organoid culture of tracheal and lung epithelial cells collected from both young and old HFD-fed mice showed higher CFE compared to SD-fed mice. Switching the HFD to low calorie/fat diet (LCD) efficiently reversed several of the HFD-induced effects. Thus, HFD induces several histological, inflammatory, and functional changes in the lung, and exacerbates the aging-induced lung inflammation and mitochondrial deterioration. LCD can reverse many of the HFD-induced effects.

1. Introduction

Aging is associated with several intrinsic and extrinsic alterations in lung cells. These alterations include cellular senescence, genomic instability, dysregulated nutrient sensing, mitochondrial dysfunction, and abnormal stem cell function (Meiners et al., 2015). The mechanisms that accelerate aging of the lung need to be elucidated. A high-fat diet (HFD) is known to shorten lifespan and to increase incidences of several metabolic diseases, including type-2 diabetes and various cardiovascular diseases (Lee et al., 2015). The diet content and the rate of weight gain seem to exert a larger influence on lifespan than the actual weight of the organism, as high saturated fat/low carbohydrate diet shortened the lifespan of mice irrespective of their body weight (Muller et al., 2013). In addition, gaining weight over a short period of time negatively influenced mice lifespan more than the body weight itself (Wagener et al., 2013). Nutrient-sensing pathways, such as the mTOR and SIRT pathways, have been shown to regulate the cellular metabolism in response to abundance/restriction of calories, and thus to be involved in the pathogenesis of several diet-related diseases (Efeyan et al., 2015). A population-based case-control study to investigate the association between dietary fat and lung cancer found that smokers with regular intake of HFD and cured meats possess an increased risk of lung cancer (Goodman et al., 1992). The mechanism by which HFD predisposes humans to lung cancer is unknown, but a recent study has

Abbreviations: AT2, alveolar type-2; BALT, bronchus-associated lymphoid tissues; CR, Calorie restriction; CFE, colony forming efficiency; H&E, hematoxylin and eosin; HFD, High fat diet, used as (HF) in figures and table 7; LNEP, lineage negative epithelial progenitor; LCD, low calorie diet; MTECs, mouse tracheal epithelial cells; ROS, reactive oxygen species; SDHA, Succinate dehydrogenase complex, subunit A; SD, standard diet; Sftpc/SP-C, surfactant protein C; WLCs, whole lung cells; VALT, vascular-associated lymphoid tissues

☆ This work was supported by JSPS Grant-in-Aid for Scientific Research (A.E.H. no. 17K09666, and M.O. no. 18K15934). The funding source had no role in design; collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

* Corresponding author at: Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Shinjuku Ku, Shinanomachi 35, Tokyo 160-8582, Japan.
E-mail address: ahegab@a2.keio.jp (A.E. Hegab).
[1] These authors contributed equally.

https://doi.org/10.1016/j.scr.2018.10.006
Received 29 June 2018; Received in revised form 26 September 2018; Accepted 2 October 2018
Available online 04 October 2018

shown that HFD induced the tumorigenicity of mice intestinal epithelial cells *via* an effect on intestinal stem cells, probably through the induction of the PPAR-δ signature in intestinal stem and progenitor cells (Beyaz et al., 2016). This is in agreement with the proposed mechanisms of stem cell decline with aging (Liu and Rando, 2011). Aging causes stem cells to lose their lineage specificity and give rise to non-functional progeny, resulting in the loss of tissue integrity and the decline of physiological function, even though the number of stem cells might remain unaffected. Stem cells might also lose their capacity to self-renew, resulting in the production of terminally differentiated daughter cells with gradual depletion of stem cells. Other known consequences of aging include senescence of stem cells or accumulation of mutations leading to uncontrolled proliferation and malignant transformation (Liu and Rando, 2011). The specific effect of aging on lung stem cells is largely unknown. Alveolar type (AT) 2 cells, the stem cell of the alveolar compartment, have been shown to undergo more extensive loss and delayed repair in old mice compared to those in young mice after influenza viral infection (Yin et al., 2014). Airway epithelial basal cells, the stem cells of the tracheal and proximal airway epithelia, were significantly less in number in old mice compared to that in young mice (Wansleeben et al., 2014). The potential harmful lung-specific effects that might be induced by HFD and the accompanying weight gain, and the mechanism by which they are associated with aging, has not been studied previously. In this work, we studied the effects of aging on mouse tracheal and alveolar epithelial stem and non-stem cells. We evaluated whether a short-term HFD will negatively influence the lung. We also compared the differential effect of initiating the HFD in old mice *versus* young mice.

2. Material and methods

2.1. Mice

Mice expressing GFP in cells expressing surfactant protein C (Sftpc/SP-C)(CBA/Ca x C57BL6J) (from Brigid Hogan) were used for the HFD study. C57BL6J naïve mice were also used for the collection of fibroblasts, some old mice, and for cell sorting when GFP fluorescence was not required. Animal experiments were approved by the Institutional Animal Care and Use Committee of Keio University.

2.2. High-fat and standard chow contents and the HFD protocol

All mice were kept on the standard chow (CE-2, Clea Japan) diet (standard diet (SD)) *ad libitum* from weaning till the start of the HFD protocol. They were then split into two groups: control SD group, in which mice were continued on the standard chow, and HFD group, in which mice were switched on to a high-fat chow (HFD32, Clea Japan) *ad libitum.* High-fat chow contained 63% more calories/unit weight than the standard chow (see supplementary tables (Meiners et al., 2015) 5 for detailed comparison of chows' contents). Each group contained 10–15 mice, and the whole HFD protocol was repeated three times. The standard chow we used is similar in formulation to the 5K0Q and the LabDiet 5 K52/5 K67, commonly used at the Jackson Laboratories, and contains 21.2% protein, 6.7% fat, and 51.3% carbohydrates in comparison to the Jackson Laboratories' chow, which contains 19.3% protein, 7.3% fat, and 53.5% carbohydrates, *i.e.*, carbohydrates are the main source of calories. On the other hand, the HFD, in addition to containing 63% higher calories/unit weight, it drove only 36.3% of its calorie contents from carbohydrates, whereas 32% of calories came from fat, and 31.7% from protein (Supplementary Table 2). Both SD and HFD were fortified with similar quantities of vitamins and minerals, which were also similar to those supplied in the chow used at the Jackson Laboratories (Supplementary Tables 3 and 4). The young mice were between 8 and 12 weeks old at the start of HFD, while the old mice were over 12 months old. Body weights of all mice were measured and recorded weekly or bi-weekly.

2.3. Lung and blood collection and tissue histology

Mice were anesthetized and exsanguinated as previously described (Hegab et al., 2015a). In brief, the thoracic cage was opened, heart and lungs were exposed, and > 500 μL of blood was collected into a heparinized syringe. Serum was collected by centrifuging the blood for 15 min at 3000 rpm. The trachea was cannulated (21G) as low as possible and secured with a thread; lungs were inflated with 4% paraformaldehyde then resected *en bloc*. The tracheal part proximal to the thread was also collected and embedded in paraffin. Paraffin-embedded lungs and tracheas were sectioned (6 μm). Sections were stained with hematoxylin and eosin (H&E) to identify histological changes. Sections were also stained with cell-type specific antibodies. Measurement of trachea epithelial thickness and nuclear densities, and the quantification of the percentage of cell types were performed as previously described (Kuroda et al., 2017). The primary antibodies used were goat SFTPC (100×, Santa Cruz), rabbit SFTPC (200×, Millipore, Temecula, CA, USA), goat CC10 (200×, Santa Cruz), mouse acetylated β-tubulin (1200×, Sigma), rabbit K5 (200×, Covance/Biolegends), rat CD45, CD3, and B220 (all 200×, Biolegends). The appropriate AlexaFluor-coupled secondary antibodies were used in double and triple stained sections. The nuclei were stained with DAPI and the slides were then examined by fluorescence microscopy using a Zeiss AxioImager microscope (Carl Zeiss). Blood serum samples were examined for total cholesterol, LDL-C, HDL—C, glucose, and NEFA (Oriental Yeast Co., ltd.).

2.4. Lung digestion and collection of different cell types

Lung digestion and the collection of different cell types was conducted as previously described (Hegab et al., 2015a; Kuroda et al., 2017). In brief, lung lobes were collected from 4 to 6 mice/group and finely minced with scissors. These samples were then incubated in Liberase (Roche)/Dispase (BD) enzyme mixture for 10 min at 37 °C. The solution was then passed through a 21G needle (four times) and filtered on a 100 μM mesh. DNase (Worthington) was added to the mesh to help release entangled cells. Hematopoietic and endothelial cells were depleted by staining the cells with CD31 and CD45 MACS beads and sorting on a manual MACS column or on AutoMACS machine (Miltenyi Biotec). For fibroblast collection, $CD45^-CD31^-$ cells were incubated in RPMI (Invitrogen) supplemented with 10% fetal bovine serum at 37 °C, 5% CO_2, and 95% air, in T75 flask for 2 h. The attached cells are the fibroblasts and the unattached cells are the whole lung epithelial cells (WLCs). WLCs were used for *in vitro* culture, or were run on a flow cytometer to sort the GFP^+ *i.e.* AT2 cells.

2.5. PCR and quantitative real-time PCR

Total RNA was extracted from whole lung tissues using the RNeasy kit (Qiagen) according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized from total RNA, using the High Capacity RNA to cDNA kit (ThermoFisher Scientific). The RT-PCR reaction was prepared using the SYBR FAST ABI Prism qPCR Kit (Kapa Biosystems) according to the manufacturer's protocol. Gene expression levels were analyzed on the StepOne Plus Real-Time PCR System (Applied Biosystems) and the 7500 Fast Real-Time PCR System (Applied Biosystems). Mouse *β-actin* was used as the endogenous control for normalization. Primers used for all genes are shown in Supplementary Table 6.

2.6. In vitro three-dimensional organoid colony cultures

Freshly collected fibroblasts (5×10^4 cells) were co-cultured with 5×10^4 WLCs or sorted AT2 cells in a 2:1 growth factor-reduced Matrigel® (BD Biosciences), with 150 μL placed in each transwell. MTEC/Plus medium (600 μL) was added to the lower chamber and