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Regulation of fatty acid synthesis and Δ9-desaturation in senescence of human fibroblasts

Miho Maeda, Natalia Scaglia *, R. Ariel Igal *

Department of Nutritional Sciences and Rutgers Center for Lipid Research, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901, USA

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ABSTRACT

Aims: Normal human cells in culture progressively lose their capacity for replication, ending in an irreversible arrested state known as replicative senescence. Senescence has been functionally associated to the process of organismal ageing and is also considered a major tumor-suppressing mechanism. Although a great deal of knowledge has uncovered many of the molecular aspects of senescence, little is known about the regulation of lipid synthesis, particularly the biosynthesis and Δ9-desaturation of fatty acids, during the senescence process.
Main methods: By using immunoblotting and metabolic radiolabeling, we determined the senescence-associated changes in major lipogenic pathways.
Key findings: The levels of fatty acid synthase and stearoyl-CoA desaturase-1 and, consequently, the formation of monounsaturated fatty acids, were notably decreased in senescent cells when compared to proliferating (young) fibroblasts. Moreover, we detected a reduction in the de novo synthesis of phospholipids with a concomitant increase in the formation of cholesterol in senescent cells compared to young fibroblasts. Finally, it was found that exogenous fatty acids were preferentially incorporated into the triacylglycerol pool of senescent cells.
Significance: This set of observations is the first demonstration of a profound modification in lipid metabolism, particularly fatty acid biosynthesis and desaturation, caused by the senescence process and contributes to the increasing body of evidence linking de novo lipogenesis with cellular proliferation.

Introduction

Normal cells exhibit an exquisite control of their life cycle by tightly regulating the events of cell mitosis and death. Unlike cancer cells in which, for the sake of endless multiplication, the mechanisms of cell replication and elimination by apoptosis are deregulated, normal cells in culture have a limited proliferative lifespan. After a finite number of divisions, cell proliferation progressively decelerates before entering a phase of irreversible growth arrest called replicative senescence (Hayflick, 2003). Other factors, such as DNA damage, activation of tumor suppressor genes, certain cell culture conditions, and even oncogenic/mitogenic stimuli, also trigger the mechanisms of senescence (Itahana et al., 2004). Senescent cells are characterized for presenting an expanded cytoplasmic compartment, a reduced capacity for DNA synthesis and the presence of senescence-associated β-galactosidase (SA-β-gal) (Dimri, 2005). Several studies have demonstrated the biological and medical relevance of the senescence process in both ageing and cancer (reviewed by Serrano and Blasco, 2007).

A mounting body of evidence supports the postulate that proliferating cells coordinate the anabolic reactions, particularly the activation of lipogenic enzymes, to provide the membrane biosynthetic machinery with lipid substrates for the making of new cellular membranes (DeBerardinis et al., 2008; Menendez and Lupu, 2007). Besides the need for greater amount of lipid structures, acyl-containing lipids, such as phospholipids, triacylglycerols (TAG), and cholesteryl esters (CE), must be composed of fatty acid species that are appropriate for supporting the structural, energetic and signaling requirements of the dividing cell. Saturated (SFA) and monounsaturated fatty acids (MUFA) account for the majority of the fatty acyl species in most mammalian cells and tissues. Given their different chemical and physical characteristics, changes in the abundance of SFA and MUFA in phospholipids, TAG and CE will determine profound alterations in the biological functions of these lipids. The precursor SFA, palmitic acid (16:0), is synthesized by fatty acid synthase (FAS) and then further elongated to stearic acid (18:0). Both palmitic and stearic acids are converted into palmitoleic and oleic acid, respectively, by stearoyl-CoA desaturases (SCDs), a family of endoplasmic reticulum-resident enzymes that catalyze the introduction of a double bond in the fatty acid carbon chain (Enoch et al., 1976). Both FAS and SCD1, the main SCD isoform in human cells (Zhang et al., 1999), are upregulated by growth factors as part of the global activation of lipogenesis during cell growth (Menendez and Lupu, 2007; Demoulin et al., 2004; Ntambi and Miyazaki, 2003). Several

* Corresponding authors. Scaglia is to be contacted at Tel.: +1 732 932 2826. Igal, Tel.: +1 732 932 9717; fax: +1 732 932 6837.
E-mail addresses: scaglia@aesop.rutgers.edu (N. Scaglia), igal@aesop.rutgers.edu (R.A. Igal).

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studies also support a role for both FAS and SCD1 in cellular proliferation. Thus, it was observed that inhibition of FAS or SCD1 in cancer cells alters lipogenesis and impairs cell mitogenesis (Menendez and Lupu 2007; Scaglia et al., 2005, Scaglia and Igal, 2005, 2008; Kuhajda et al., 1994), suggesting that both de novo synthesis of fatty acids and their conversion to MUFA are necessary for cell proliferation. Moreover, in other cellular processes that require membrane synthesis independently of cell proliferation, like phagocytosis, de novo lipogenesis is also increased (Castoreno et al., 2005; Fagone et al., 2007). As a whole, these data suggest that except for those tissues specialized in lipid storage or secretion, lipogenesis is tightly coupled to membrane synthesis. With the aim of searching for more evidence to validate this hypothesis, we sought to determine whether the contrary is also true, i.e., if de novo lipogenesis is reduced when the net demand for membrane lipids decreases.

Cells undergoing senescence progressively lose their ability to divide, but remain metabolically active in their quiescent state. However, very little is known about the regulation of lipid metabolism, especially lipid biosynthesis, in this physiological process. The goal of the present study was to investigate the regulation of lipid synthesis, particularly focusing on the de novo synthesis and Δ9-desaturation of fatty acids, in a model of cellular senescence.

Materials and methods

Materials

AG01518 normal human skin fibroblasts were obtained from Coriell (Camden, NJ). Cell culture media and other culture reagents were purchased from Invitrogen Life Technologies (Carlsbad, CA). [1-^{14}C] Stearic acid and [6-^{3}H]thymidine were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Ultrafiltered fetal bovine serum (FBS), fatty acid-free bovine serum albumin (BSA), mouse anti-β-actin monoclonal antibody, anti-mouse IgG peroxidase conjugate, phosphatase and protease inhibitor cocktail were purchased from Sigma (St. Louis, MO). Pure lipid standards were from Doosan Serdary (Yongin, Korea) and NuCheck Prep, (Elysian, MN). Cell culture supplies, silica gel 60 chromatography plates, and analytical-grade solvents were purchased from Fisher Scientific, (Morris Plains, NJ). Polyclonal anti-SCD1 antibody was a generous gift of Dr Jean-Baptiste Demoulin, Université Catholique de Louvain, Belgium. Monoclonal anti-FAS antibody was from Abcam (Cambridge, MA).

Cell culture

Fibroblasts were routinely cultured in 100 mm Petri dishes in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% FBS, 1% vitamins, penicillin (100 U/ml), streptomycin (10 μg/ml), and 1% non-essential amino acids at 37 °C, and 5% CO_2.

Western blot

Total cellular homogenates were obtained by lysis in ice-cold hypotonic buffer with protease inhibitors. Proteins were resolved by SDS-PAGE and transferred onto a nitrocellulose membrane. Membranes were then probed with primary antibodies targeted against SCD1, FAS, and β-actin in 1:1000 dilutions. After incubation with appropriate secondary antibodies, bands on the membrane were visualized using a chemiluminescence detection kit and were quantified using a Molecular Imager ChemiDoc XRS system (BioRad Laboratories, Hercules, CA).

Determination of cellular protein

Total cellular protein content was measured by Bradford method using Pierce Coomassie Plus Protein Assay Reagent, with BSA as a standard.

Senescence assay

The presence of senescence associated β-galactosidase (SA-β gal) was determined as described by Dimri et al. (1995). Briefly, the fibroblasts were grown to 80–90% confluence, washed with phosphate saline buffer (PBS) and fixed in 3% paraformaldehyde. The cells were stained with β-gal staining solution containing 1 mg/ml 5-bromo-4-chloro-3-indoyl β-D-galactopyranoside (X-gal) at 37 °C for an additional 12–16 h and then photographed under a microscope.

Metabolic labeling and lipid extraction

Preconfluent cells (80–90% confluence) were incubated with trace amounts (2.3 μM) of [^{14}C]stearic acid (0.25 μCi/dish) or [^{14}C] acetate (0.50 μCi/dish) in culture medium containing 0.5% BSA for up to 24 h. At the end of the labeling period, cell lipids were extracted following the procedure of Bligh and Dyer (1959) and radioactive lipids were analyzed as described in Scaglia and Igal (2005). The amount of radioactivity incorporated into each lipid class was calculated from the specific activity of each substrate and normalized to cellular protein content.

Determination of SCD activity in cells

The Δ9-desaturation of fatty acids in non-senescent and senescent cells was assessed as previously described (Scaglia and Igal, 2005).

[^{3}H]Thymidine incorporation into cell DNA

The rate of DNA synthesis was estimated by determining the levels of [^{3}H]thymidine incorporation into DNA after pulsing the cells with the radiolabeled tracer (1 μCi/dish) for up to 4 h, followed

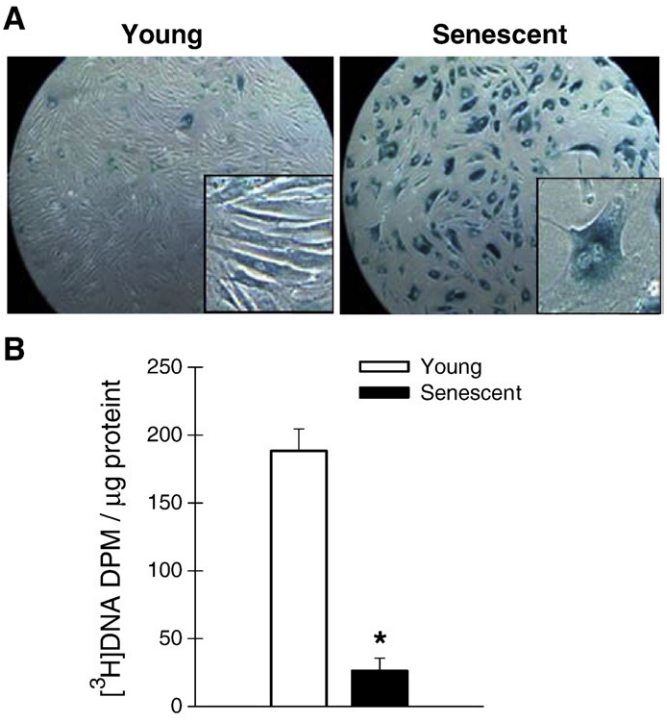

Fig. 1. Determination of senescence in human skin fibroblasts. Panel A, senescence was estimated by detection of SA-β-galactosidase activity in cells at passages 15 (young) and 25 (senescent). Insets, visualization of cells in a microscopic field. Panel B, young and senescent cells were pulsed with [^{3}H]thymidine (1 μCi/dish) in 10% FBS, DMEM for 2 h and 37 °C. Total [^{3}H]-labeled DNA was precipitated and radioactivity was quantified in a scintillation counter. Values correspond to the mean ± S.D. of triplicate dishes. *, $P<0.01$, by Student's t test.