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Short report

Depletion of oxaloacetate decarboxylase FAHD1 inhibits mitochondrial electron transport and induces cellular senescence in human endothelial cells

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ABSTRACT

In this study we report the identification of FAH domain containing protein 1 (FAHD1), a recently described member of the fumarylacetoacetate hydrolase (FAH) superfamily of metabolic enzymes, as a novel player in the regulation of cellular senescence. FAHD1 was found in a proteomic screen searching for mitochondrial proteins, which are differentially regulated in mitochondria from young and senescent human endothelial cells, and subsequently identified as oxaloacetate decarboxylase. We report here that depletion of FAHD1 from human endothelial cells inhibited mitochondrial energy metabolism and subsequently induced premature senescence. Whereas senescence induced by FAHD1 depletion was not associated with DNA damage, we noted a reduction of mitochondrial ATP-coupled respiration associated with upregulation of the cdk inhibitor p21. These results indicate that FAHD1 is required for mitochondrial function in human cells and provide additional support to the growing evidence that mitochondrial dysfunction can induce cellular senescence by metabolic alterations independent of the DNA damage response pathway.

1. Introduction

Cellular senescence is increasingly recognized as a physiological process involved in a variety of physiological and pathophysiological conditions in mammals. Thus, cellular senescence contributes to tumor suppression (Campisi, 2011), age-associated dysfunction (Campisi, 2013; van Deursen, 2014), and mammalian development (Burton and Krizhanovsky, 2014; Munoz-Espin et al., 2013). Senescent cells express a number of so-called senescence markers, including the cell cycle inhibitors $p16^{INK4A}$ and $p21^{Cip1/Waf-1}$, as well as increased activity of senescence-associated β-galactosidase (Rodier and Campisi, 2011). Many senescent cells also secrete several cytokines, growth factors, and matrix metalloproteinases, collectively referred to as the senescence-associated secretory phenotype (SASP; (Coppe et al., 2008; Malaquin et al., 2016). In addition to these "classical" senescence markers, recent evidence suggests that senescence-associated metabolic changes, including altered mitochondrial function, may be essential for the induction and maintenance of the senescent state (Nacarelli and Sell, 2016).

Whereas replicative senescence and several forms of stress-induced senescence are characterized by DNA damage at telomeres and other genomic loci, in particular DNA double strand breaks, other types of senescence have been characterized that occur in the absence of DNA damage (Bhatia-Dey et al., 2016). Thus, developmental senescence (Munoz-Espin et al., 2013), senescence in response to a lowered ATP/AMP ratio (Zwerschke et al., 2003), and senescence induced by inhibition of the mitochondrial electron transport chain (ETC) (Stockl et al., 2006; Wiley et al., 2016) are not accompanied by detectable DNA damage.

Besides human diploid fibroblasts which are still used in the vast majority of in vitro senescence studies, many other human cell types also display the senescence phenotype upon extended passaging. Thus, human umbilical vein endothelial cells (HUVEC) were shown to enter cellular senescence after roughly 60 population doublings (Wagner et al., 2001). As in fibroblasts, premature senescence can be induced in HUVEC by a variety of stimuli, including exposure to tert-Butyl-hydroperoxide (t-BHP) (Unterluggauer et al., 2003), which leads to induced oxidative stress. Of note, replicative senescence of HUVEC could be delayed by silencing the gene encoding NADPH oxidase NOX4 (Lener et al., 2009), indicating that H_2O_2 produced by endogenous NOX4, induces nuclear DNA damage and thereby accelerates senescence in this cell type. However, HUVEC senescence is also induced

Abbreviations: BrdU, 5-bromo-2′deoxy-uridine; ETC, electron transport chain; FAHD1, fumarylacetoacetate hydrolase (FAH) domain-containing protein 1; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; HUVEC, human umbilical vein endothelial cells; JC-1, 5.5′,6,6′-tetrachloro-l,l′,3,Y-tetraethylbcnzimidazolocarbocyanine iodide; MMP, mitochondrial membrane potential; OAA, oxaloacetate; OCR, oxygen consumption rate; PI, propidium iodide; SA-β-Gal, senescence-associated β-galactosidase; SCR, scrambled; shRNA, short hairpin RNA; TCA cycle, tricarboxylic acid cycle.

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by the inhibition of glutaminase (Unterluggauer et al., 2008) which affects ATP production and other biosynthetic pathways based on glutaminolysis. However, the role of mitochondria in HUVEC senescence remained elusive.

In an attempt to identify new mitochondrial regulators of cellular senescence, a proteomics approach was used to identify mitochondrial proteins, the expression of which is altered with cellular senescence, using HUVEC as model. Besides post-translationally modified isoforms of several mitochondrial ATPase subunits (Groebe et al., 2007), we also identified fumarylacetoacetate hydrolase (FAH) domain containing protein 1 (FAHD1) as a differentially expressed protein in mitochondria from young vs. senescent HUVEC. Subsequently, FAHD1 was functionally characterized as a mitochondrial metabolic enzyme with dual substrate specificity, which can both hydrolyze acylpyruvates (Pircher et al., 2011) and decarboxylase oxaloacetate (Pircher et al., 2015), a metabolite of the tricarboxylic acid (TCA) cycle. When the FAHD1 homolog *fahd-1* was deleted in the nematode *C. elegans*, this resulted in a severe locomotion deficit which was accompanied by a significant reduction of both mitochondrial membrane potential and oxygen consumption (Taferner et al., 2015), suggesting that FAHD1 is required for mitochondrial function in nematodes. However, the role of FAHD1 in mammalian cells remained elusive. In the present communication, we have explored the effects of lentivirus mediated silencing of the FAHD1 gene in HUVEC.

2. Material and methods

2.1. Cell culture

HUVEC were isolated and maintained according to the methods described (Wagner et al., 2001). Cells were propagated in endothelial cell growth medium (EBM CC-3121 supplemented with CC-4133, Lonza, Walkersville, USA). U-2OS and HeLa cells purchased from ATCC (Manassas, VA) were propagated in Dulbecco's modified Eagle's medium (D5546, Sigma, Steinheim, Germany) supplemented with 10% heat-inactivated fetal bovine serum (Sigma, Steinheim, Germany), 4 mM L-glutamine (Sigma, Steinheim, Germany), and 1% penicillin streptomycin (Gibco, Eggenstein, Germany). 293FT cells were grown and maintained according to the supplier's user manual (Cat. nos. R700-07, WFGE08S, Invitrogen). All cells were grown in an atmosphere of 5% CO_2 at 37 °C and were subcultured by trypsinization with 0.05% trypSsin-EDTA (Sigma, Steinheim, Germany).

2.2. Metabolic flux analysis

Metabolic flux analysis was performed using Seahorse XFp Analyser (Seahorse Bioscience, North Billerica, MA). Shortly, 5×10^3 cells/well were seeded out one day before the experiment on the XFp cell culture miniplates (Seahorse Bioscience, North Billerica, MA) and analysed according to the protocols provided by the manufacturer. The Seahorse XFp Cell Mito Stress Test Kit (Seahorse Bioscience, North Billerica, MA, # 103010-100) was used. Obtained data were analysed using Wave 2.3.0 software (Agilent Technologies).

2.3. Determination of mitochondrial membrane potential

The electric potential of the inner mitochondrial membrane was determined in the cells pre-stained with the JC-1 fluorescent probe (Thermo Fisher Scientific, Vienna, Austria), as described previously (Koziel et al., 2013). Fluorescence was measured using the FACS Canto II flow cytometer (Becton Dickinson, Heidelberg, Germany).

2.4. Preparation of cellular extracts and western blot

Cellular protein lysates were prepared in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and 0.1% SDS) and subjected to standard immunoblotting, using primary antibodies against FAHD1 (rabbit polyclonal, dilution 1:1000, desribed in Pircher et al., 2011), α-tubulin (Cat.-No. T5168, dilution 1:20,000, Sigma, Vienna, Austria), p53 (Cat.-No. SC-126, dilution 1:000, Santa Cruz Biotechnology, Heidelberg, Germany), p21 (Cat.-No. 2947, dilution

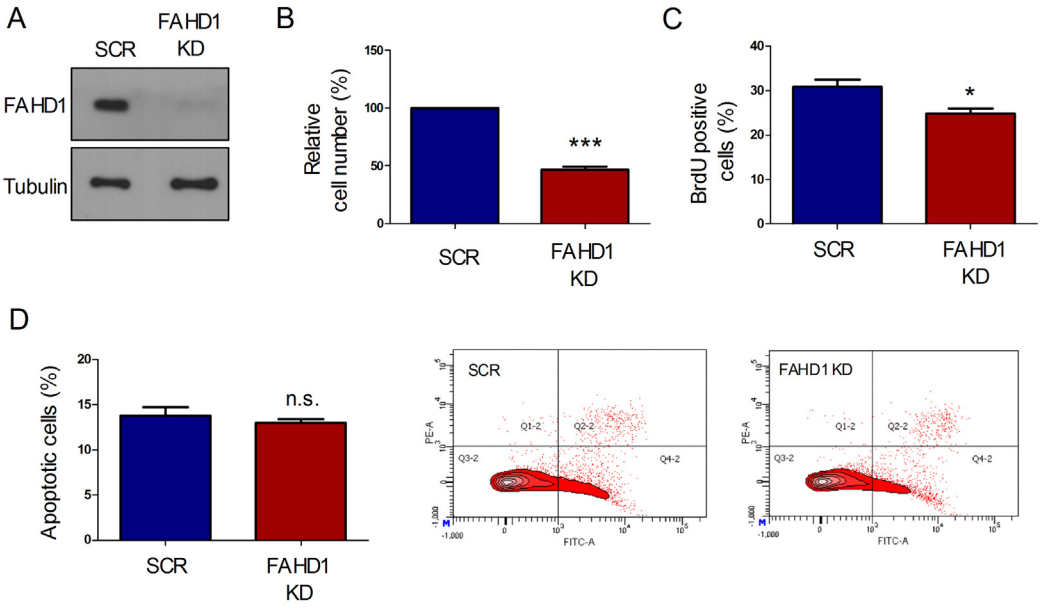

Figure 1

Fig. 1. Downregulation of FAHD1 reduces cell proliferation in human endothelial cells. Early-passage HUVEC, were infected with 1 MOI of control (SCR) or FAHD1 knockdown lentivirus, as indicated, and FAHD1 levels determined by Western blot; α-tubulin served as loading control (A). Subsequently, cells were counted seven days after infection (B), and the rate of cell proliferation determined by BrdU incorporation assay (C). Cell death was assessed by co-staining with Annexin V and PI followed by flow cytometry (D).