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Extracellular reactive oxygen species are generated by a plasma membrane oxidative phosphorylation system

Hyun Lee[a,b,1], Bong-Woo Kim[a,b,1], Jung-Woo Lee[a,b], Jin Hong[a,b], Jung-Wha Lee[c], Hong-Lim Kim[d], Jae-Seon Lee[e], Young-Gyu Ko[a,b,*]

[a] Division of Life Sciences, Korea University, Seoul, Republic of Korea
[b] Tunneling Nanotube Research Center, Korea University, Seoul, Republic of Korea
[c] Department of Biochemistry, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[d] Integrative Research Support Center, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[e] Department of Biomedical Sciences, College of Medicine, Inha University, Incheon, Republic of Korea

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ABSTRACT

Although the oxidative phosphorylation (OXPHOS) system has been found in mitochondria and the plasma membrane of various mammalian cell lines, understanding the physiological functions of the plasma membrane OXPHOS system is challenging. Here, we demonstrated that OXPHOS I, II, III, IV and V subunits were expressed in the plasma membrane of HepG2 cells and primary mouse hepatocytes, as determined by non-permeabilized immunofluorescence, total internal reflection fluorescence (TIRF) microscopy, cell surface-biotin labeling and plasma membrane and lipid raft isolation. Next, we demonstrated that NADH administration generated extracellular superoxide and improved insulin signaling in HepG2 cells and primary mouse hepatocytes. The NADH-dependent generation of extracellular superoxide was prevented by knockdown of NDUFV-1, the first subunit of OXPHOS I receiving electrons from NADH and the NADH-improved insulin signaling was abolished by extracellular catalase. Thus, we conclude that the OXPHOS system in the plasma membrane may be required for the generation of extracellular ROS and the regulation of insulin signaling.

1. Introduction

Protein moonlighting refers to the idea that a specific proteins have multiple functions, depending on their partners, oligomer formation, cellular location and expression level [1,2]. Moonlighting protein activities might derive from ancient enzymatic functions. For example, CLOCK-1 (CLK-1) is a demethoxyubiquinone monooxygenase for ubiquinone biosynthesis in the mitochondria [3]. CLK-1 has an unexpected function that regulates gene expression associated with the mitochondrial unfolded protein response after mitochondrial to nuclear translocation in the presence of increased mitochondrial reactive oxygen species (ROS) [3–5]. Cytochrome *c*, a crucial component of the mitochondrial oxidative phosphorylation (OXPHOS) system, translocates from the mitochondria to the cytoplasm and activates caspase-9 when apoptosis is stimulated [6–8]. In addition, signal transducer and activator of transcription 3 (STAT3) translocates from the nucleus to the mitochondria and participates in the mitochondrial OXPHOS system [9,10].

The mitochondrial OXPHOS system is the canonical producer of adenosine triphosphate (ATP) and is composed of NADH-coenzyme Q oxidoreductase (complex I), succinate-Q oxidoreductase (complex II), Q-cytochrome *c* oxidoreductase (complex III), cytochrome *c*, cytochrome *c* oxidase (complex IV), and ATP synthase (complex V). During oxidative phosphorylation, the electron transport chain transfers electrons from NADH or $FADH_2$ to O_2, forming a proton gradient across the mitochondrial inner membrane. Finally, ATP synthase generates ATP by coupling a proton gradient to ADP phosphorylation. Unexpectedly, different OXPHOS subunits have also been found in the sarcolemma from C2C12 myotubes and mouse gastrocnemius [11–14]. The sarcolemma OXPHOS system is necessary for NADH-dependent generation of extracellular reactive oxygen species (exROS), consuming oxygen [13].

ROS regulate diverse intracellular signaling pathways, depending on different biological targets. As signaling molecules, ROS regulate cellular proliferation, activate the inflammatory response in damaged tissue and control stress response and longevity [15–18]. Especially, ROS selectively oxidize cysteine residues of proteins such as protein-tyrosine phosphatase (PTP) and PTEN. Thus, the inactivation of these phosphatases enhances signaling pathways such as PI3K-Akt and Ras/

* Corresponding author at: Division of Life Sciences, Korea University, 145, Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea.
E-mail address: ygko@korea.ac.kr (Y.-G. Ko).
[1] Both authors contributed equally to this work.

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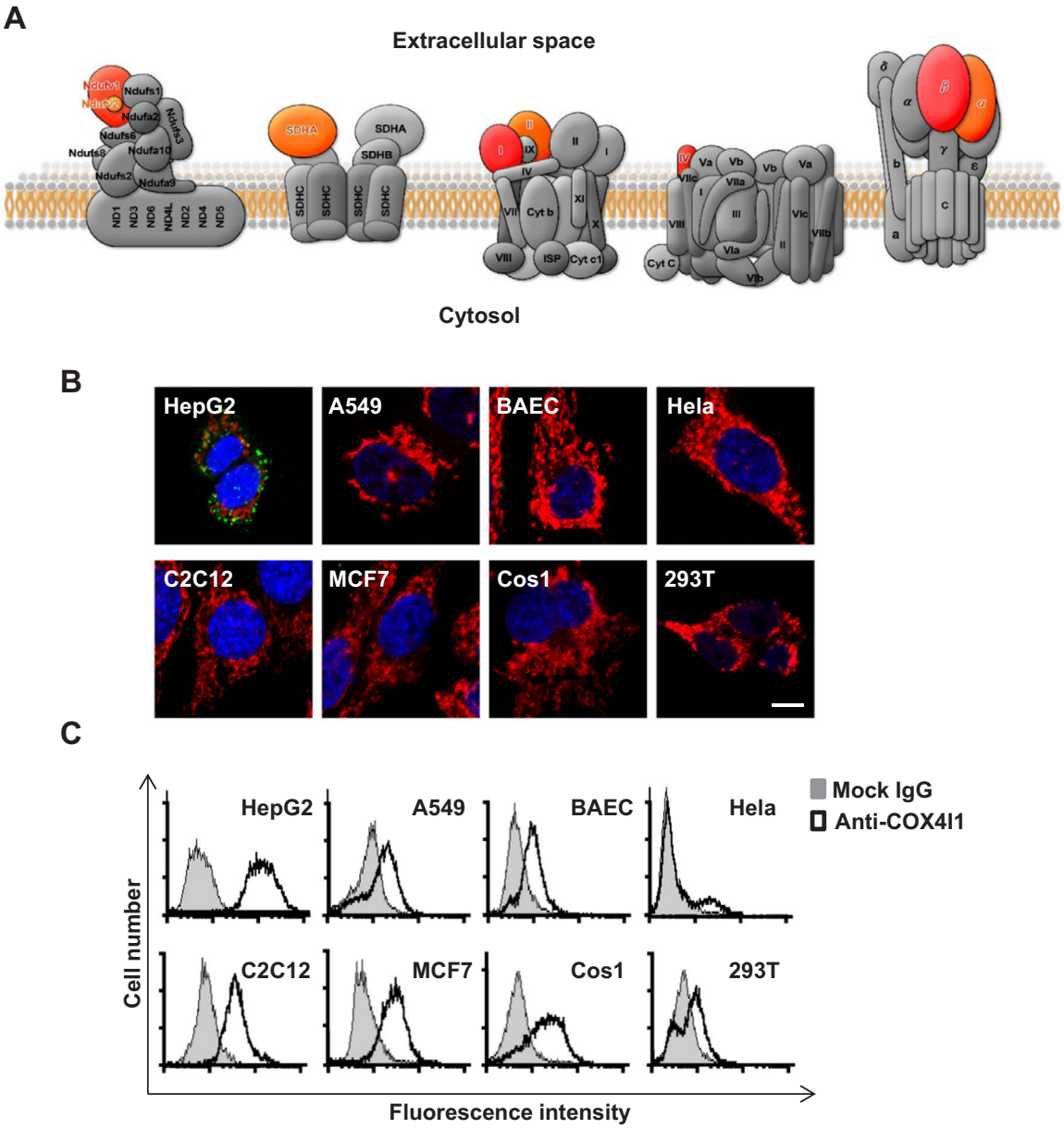

Fig. 1. The cell-surface expression of OXPHOS IV on a variety of cell lines. (A) Topology of plasma membrane OXPHOS system. The colored part of each OXPHOS system represents a subunit that faces the extracellular space. The colored subunit in each OXPHOS system was selected for non-permeabilized immunofluorescence. OX, oxidative phosphorylation complex; NDUFV1, NADH dehydrogenase [ubiquinone] flavoprotein 1; NDUFV2, NADH dehydrogenase [ubiquinone] flavoprotein 2; SDHA, succinate dehydrogenase complex flavoprotein subunit A; UQCRC1, ubiquinol-cytochrome *c* reductase complex core protein 1; UQCRC2, ubiquinol-cytochrome *c* reductase complex core protein 2; COX4I1, cytochrome *c* oxidase subunit 4 isoform 1; ATP5A, ATP synthase α; ATP5B, ATP synthase β□ (B) Non-permeabilized immunofluorescence of COX4I1 (a subunit of OX IV) in various mammalian cells. HepG2, A549, bovine aorta endothelial cell (BAEC), HeLa, C2C12 myoblasts, MCF7, COS-1 and 293T cells were stained with an anti-COX4I1 antibody, Mito-Tracker Red CMXRos and DAPI without cell permeabilization. Scale bar = 5 μm. (C) Non-permeabilized mammalian cells were immunostained with an anti-COX4I1 antibody for FACS analysis.

MEK/ERK cascades [19]. Mice lacking the antioxidant glutathione peroxidase show higher ROS levels, leading to inactivation of PTEN and resulting in higher PI3K/Akt signaling and improving insulin sensitivity. Thus, ROS is necessary for maintaining normal insulin sensitivity [20].

In this study, we found that different OXPHOS subunits were localized in the plasma membrane of HepG2 cells and primary mouse hepatocytes. We also demonstrated that NADH treatment generated exROS and then enhanced insulin signaling using the plasma membrane OXPHOS system.

2. Results

2.1. The COX4I1 subunit was ectopically expressed in the plasma membrane of HepG2 cells

Oxidative phosphorylation IV (OXPHOS IV), also known as cytochrome *c* oxidase (COX), consists of 14 subunits. Among these subunits, cytochrome *c* oxidase subunit 4 isoform 1 (COX4I1) might face towards the extracellular space if present in the plasma membrane (Fig. 1A). To determine the expression level of COX4I1 on the plasma membrane, we performed the immunofluorescence and flow cytometry assays in a variety of non-permeabilized cells such as HepG2, A549, BAEC, HeLa, C2C12 myoblasts, MCF7, COS1, and 293T cells. As shown in Fig. 1B, we observed a punctate pattern of the COX4I1 subunit on the plasma