The International Journal of Biochemistry & Cell Biology 45 (2013) 156–166

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Targeting mitochondria in the infection strategy of the hepatitis C virus☆

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ARTICLE INFO

Article history:
Available online 16 June 2012

Keywords:
Hepatitis C virus
Mitochondria
Redox signalling
Calcium signalling
Permeability transition

ABSTRACT

Hepatitis C virus (HCV) infection induces a state of oxidative stress more pronounced than that observed in many other inflammatory diseases. Here, we propose a temporal sequence of events in the HCV-infected cell whereby the primary alteration consists of a release of Ca^{2+} from the endoplasmic reticulum, followed by uptake into mitochondria. This ensues successive mitochondrial dysfunction leading to the generation of reactive oxygen species and a progressive metabolic adaptive response. Evidence is provided for a positive feed-back mechanism between alterations of calcium and redox homeostasis. This likely involves deregulation of the mitochondrial permeability transition and induces progressive dysfunction of cellular bioenergetics. Pathogenetic implications of the model and new opportunities for therapeutic intervention are discussed.

This article is part of a Directed Issue entitled: Bioenergetic dysfunction, adaptation and therapy.

1. Introduction

Infection by the hepatitis C virus (HCV) is a major cause of chronic liver disease. Worldwide about 120–200 million people are chronically HCV-infected and at risk of developing cirrhosis and hepatocellular carcinoma (HCC) (Nature Outlook, 2011). HCV is a positive-strand RNA virus with a 9.6-kb genome, composed of 5′ and 3′ nontranslated regions flanking an open reading frame (ORF) encoding a large polyprotein, which is translated on the endoplasmic reticulum (ER) and converted by host and viral proteases into ten individual ER-associated viral proteins (Moradpour et al., 2007). The structural proteins core, E1 and E2 build up the virus particle whereas the p7 polypeptide and the nonstructural proteins (NS2-NS5B) are required for virion assembly and RNA replication (Moradpour et al., 2007).

Evidence indicates that HCV infection causes ER stress and the unfolded protein response (UPR) (Asselah et al., 2010; Merquiol et al., 2011; Tardif et al., 2005), Ca^{2+} homeostasis deregulation (Korenaga et al., 2005; Li et al., 2007a,b; Piccoli et al., 2007) and reactive oxygen species (ROS) production by mitochondria (Piccoli et al., 2006, 2007; Wang and Weinman, 2006). In this review article, we combine recent literature with evidence provided by our group to highlight mitochondria as a target during HCV infection. A novel pathogenetic model for HCV-related disease will be put forward along with suggestions for potential therapeutic interventions.

2. Mitochondria: a brief overview of their functions affected by HCV infection

Mitochondria constitute the powerhouse of the cell producing up to 90% of the ATP depending on cell type and/or metabolic conditions (Saraste, 1999). This stands, particularly, for cardiomyocytes, neurons and other high energy-demanding cells in aerobic

Abbreviations: CARD, caspase activation and recruitment domain; CARDIF, CARD adaptor inducing intereferon-β; CAT, carnitine acyl-CoA transferase; ER, endoplasmic reticulum; FFA, free fatty acid; GSH, reduced glutathione; GSSG, oxidised glutathione; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIF-1, hypoxia-inducible factor 1; IMM, inner mitochondrial membrane; IPS-1, interferon-β promoter stimulator 1; MAMs, mitochondria-associated membranes; MPTP, mitochondrial permeability transition pore; mt-Ca^{2+}, intramitochondrial Ca^{2+}; mt$\Delta\Psi$, mitochondrial electrical membrane potential; NAC, N-acetylcysteine; OMM, outer mitochondrial membrane; OXPHOS, oxidative phosphorylation; RC, respiratory chain; RIG-1, retinoic acid inducible gene-1; RNS, reactive nitrogen species; ROS, reactive oxygen species; RyR, ryanodine receptor; SERCA, sarco/endoplasmatic reticulum calcium ATPase; SREBP-1c, sterol regulatory element-binding protein; UPR, unfolded protein response; VISA, virus-induced signaling adapter; $\Delta\mu H^+$, electrochemical transmembrane potential.

☆ This article is part of a Directed Issue entitled: Bioenergetic dysfunction, adaptation and therapy.

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http://dx.doi.org/10.1016/j.biocel.2012.06.008

tissues/organs. Electron transfer (from NADH- and $FADH_2$-linked substrates) through the respiratory chain (RC) complexes I to IV, localized in the inner mitochondrial membrane (IMM), ultimately reduces O_2 to water. The large free energy thereby made available is utilized to pump protons, thus generating an electrochemical transmembrane potential ($\Delta\mu H^+$, 180–200 mV, positive outside) which is exploited to generate ATP by the F_oF_1 H^+-ATP-synthase and to drive other endoergonic processes (Saraste, 1999).

Although providing high ATP yield, the drawback of the aerobic metabolism is the risk of single-electron transfer from the redox centers-containing RC complexes directly to O_2, with formation of the superoxide anion radical ($O_2^{\bullet -}$) (Turrens, 2003). Under physiological conditions, up to 1-2% of the O_2 consumed was reported to be converted into ROS (Boveris, 1984). This estimate has been recently downsized (Rigoulet et al., 2011). However, a number of circumstances have been described leading to enhanced $O_2^{\bullet -}$ generation. They comprise events either accelerating or retarding the electron flow through the RC complexes and some of those have physio-pathological relevance (Turrens, 2003; Fruehauf and Meyskens, 2007). $O_2^{\bullet -}$ is the seminal ROS which gives rise to formation of the freely diffusible H_2O_2 and $OH^{\bullet}$ (by a Fenton-like reaction). Generation of $O_2^{\bullet -}$ along with $NO^{\bullet}$ may result in formation of peroxynitrite and other reactive nitrogen species (RNS) (Wink and Mitchell, 1998). The harmful potential of ROS and RNS is counteracted by an armory of antioxidants (enzymes and soluble or lipophilic compounds) that contribute to keep the intra-cellular ROS level as low as possible (Valko et al., 2007). Conditions causing unbalance between production and removal of ROS lead to change in the redox tone of the cell commonly referred as oxidative stress (Fruehauf and Meyskens, 2007). In this pathological context mitochondria proved to be credited in a growing number of human diseases (Lenaz, 2012).

However, unbalance of the cellular redox tone is not always associated with a pathological condition. Indeed controlled changes of the intracellular oxidation state are exploited by the cell as signals to activate a number of adaptive physiological responses (Martindale and Holbrook, 2002; Boonstra and Post, 2004; Valko et al., 2007; Finkel, 2011; Rigoulet et al., 2011; Handy and Loscalzo, 2012). The release of oxidants is interpreted as a signal that a stress had been encountered, with the intensity, duration and localization of ROS release a potential determining factor in the ultimate biological outcome. ROS are a heterogeneous family with two kinds of molecules with very different status: (a) the radical forms of oxygen that are highly reactive, toxic, and have a very short half-life; and (b) nonradical forms such as hydrogen peroxide, which is a much more stable (and membrane permeant) molecule. Consequently, hydrogen peroxide is thought to be the main form involved in ROS signaling. Hydrogen peroxide signaling can be either direct (oxidation of its target) or indirect (involving peroxiredoxins, for example). Low intensity ROS production may be important in metabolic adaptation such as seen with nutrient excess or under conditions of low oxygen as well as in controlling cell growth/proliferation and differentiation. Moderate ROS production stimulated by endogenous or exogenous danger signals might be involved in regulating inflammatory mediators. Finally, high level ROS production might signal the induction of pathways such as apoptosis or autophagy capable of inducing cell death. In each case, different redox-sensitive cytosolic pathways would be mobilized and the details of the underlining molecular mechanisms still remain to be elucidated.

Although other cellular processes unrelated to the RC activity can release considerable amount of ROS (Brown and Borutaite, 2012), mitochondria are an important source of ROS within the cell and, therefore, are likely to play a role in this context even under physiological conditions as suggested by multiple lines of evidence (Finkel, 2011; Rigoulet et al., 2011; Handy and Loscalzo, 2012).

In addition to driving ATP synthesis, the $mt\Delta\Psi$ (i.e. the electrical component of the mitochondrial $\Delta\mu H^+$) is utilizable to carry on other energy requiring processes such as ion transport. Calcium, in particular, deserves consideration, as its intracellular level controls a number of signaling pathways (Nicholls, 2005). Cytosolic Ca^{2+} rise is caused by ion leakage from the plasma membrane and/or intracellular stores and is exacerbated by a deficit in available ATP, which is needed for functioning of the Ca^{2+}-ATPase pumps (Brookes et al., 2004). Beyond certain levels Ca^{2+} is cytotoxic and under such emergency conditions mitochondria operates as a low affinity high capacity Ca^{2+}-buffering system. The main system by which mitochondria import Ca^{2+} is a $mt\Delta\Psi$-driven Ca^{2+} uniporter (Deryabina et al., 2004). The inward current of Ca^{2+} into mitochondria accomplishes a double task: it helps to normalize the cytosolic free Ca^{2+} concentration and, in addition, stimulates the terminal metabolism by activation of the Krebs' cycle dehydrogenases (Brookes et al., 2004; Deryabina et al., 2004; Rimessi et al., 2008). This elicits a larger production of reducing substrates to fuel oxidative phosphorylation (OXPHOS), thereby providing extra ATP to the Ca^{2+}-ATPase pumps.

Mitochondria play a fundamental role in controlling the cell fate. A battery of pro-apoptotic factors (cytochrome c, SMAC/DIABLO, AIF, etc.) are kept in the mitochondrial inter-membrane space and, when released by appropriate stimuli, activate caspase-dependent programmed cell death (Chalah and Khosravi-Far, 2008; Kroemer et al., 2007). However, a number of anti-apoptotic factors harbored in or directed to mitochondria (i.e. BCL-2, IAP, survivin, p53, c-myc, RAS, etc.) contribute to balance the cell fate. Therefore, mitochondria behave as sensors and coordinators of a large number of hits whose sum decides if the cell must survive or die. Among the pro-apoptotic signals are either a condition of nitro-oxidative stress and/or of mitochondrial Ca^{2+} overload. ROS and Ca^{2+} exert their action on the opening probability of the mitochondrial permeability transition pore (MPTP) (Kroemer et al., 2007) (Fig. 4). Although not completely defined from the structural/mechanistic viewpoint, MPTP plays a central role in promoting, indirectly, the diffusion across the outer mitochondrial membrane (OMM) of pro-apoptotic factors (Kroemer et al., 2007).

Finally, recent studies have uncovered a previously unsuspected role of mitochondria in antiviral innate immune responses (Castanier and Arnoult, 2011; West et al., 2011). An antiviral pathway uses the RNA helicase RIG-I as the receptor for intracellular viral RNA. RIG-I activates NF-κB and IRFs through the adaptor protein Cardif (also known as IPS-1, VISA and MAVS), a CARD-containing protein that resides in the outer mitochondrial membrane (Meylan et al., 2005). Cardif is essential for antiviral innate immunity, but is targeted by the HCV NS3-4A serine protease, which cleaves Cardif off the mitochondria, thereby enabling HCV to escape the host immune system.

3. Interaction of HCV proteins with mitochondria

In keeping with the premises above, it is not surprising that a mounting number of diseases are linked directly or indirectly to mitochondrial dysfunction (Wallace, 2005). The attention that basic and clinical investigators address to mitochondria is prompted by the hope to find novel therapeutic targets (Serviddio et al., 2010; Frantz and Wipf, 2010). Chronic hepatitis C does not escape this trend. Indeed, since the earlier findings of altered morphology in hepatic mitochondria of patients with chronic hepatitis C (Barbaro et al., 1999), a growing number of studies have focused on the involvement of mitochondria in the pathogenesis of HCV-related liver disease.

In this context, an important aspect to be considered is the intracellular compartmentalization of the HCV proteins. Although the