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Review

HCV infection induces mitochondrial bioenergetic unbalance: Causes and effects

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ABSTRACT

Cells infected by the hepatitis C virus (HCV) are characterized by endoplasmic reticulum stress, deregulation of the calcium homeostasis and unbalance of the oxido-reduction state. In this context, mitochondrial dysfunction proved to be involved and is thought to contribute to the outcome of the HCV-related disease. 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 causes successive mitochondrial alterations comprising generation of reactive oxygen and nitrogen species and impairment of the oxidative phosphorylation. A progressive adaptive response results in an enhancement of the glycolytic metabolism sustained by up-regulation of the hypoxia inducible factor. Pathogenetic implications of the model are discussed.

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1. Introduction

Infection by the hepatitis C virus (HCV) is a major cause of chronic liver disease [1]. Worldwide about 170 million people are chronically HCV-infected and are at risk of developing cirrhosis and hepatocellular carcinoma (HCC). 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 viral and host proteases into ten individual membrane-associated viral proteins [2]. 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 [2].

Evidence indicates that HCV infection causes ER stress and the related unfolded protein response (UPR) [3] as well as Ca^{2+} homeostasis deregulation [4,5]. In addition, alterations of mitochondria with inhibition of the respiratory chain (RC) and generation of reactive oxygen species (ROS) have been reported to be elicited by HCV protein expression [5–7]. In this contribution, we will combine the recent literature with evidence provided by our group to put forward a mechanistic pathogenetic model.

2. Interaction of HCV proteins with mitochondria

A mounting number of diseases are reported to be linked directly or indirectly to dysfunctioning mitochondria [8]. This is not surprising given the central role that mitochondria play not only in the cell bioenergetics [9] but also in controlling signaling networks involved in cell fate decision [10] and innate immune response [11]. The attention that basic and clinical investigators direct to mitochondria is prompted by the hope to find novel therapeutic targets. Chronic hepatitis C does not escape this trend. Indeed, since the earlier findings of altered morphology of hepatic mitochondria in patients with chronic hepatitis C [12], a growing number of studies have been focusing on the involvement of mitochondria in the pathogenesis of HCV-related liver disease.

Although the synthesis and maturation of HCV proteins occur at the level of the ER [2], a number of studies unveiled partial localization of some HCV proteins, notably core and NS3/4A, to the outer mitochondrial membrane (OMM) [13–19]. In particular, HCV proteins appear to accumulate at points of contact between mitochondria and the ER (also designated as mitochondria-associated membranes, MAMs). This led to the proposal that HCV proteins migrate to mitochondria by lateral diffusion from the ER via transient fusion of the membraneous sub-compartments [14]. Therefore, the conviction of a direct interaction of certain HCV proteins with mitochondrial membrane constituents has been progressively emerging though the mechanism of interaction remains yet elusive.

Mitochondria form a dynamic reticulum intertwined with the ER. High-resolution imaging studies proved that the two membranous sub-cellular compartments physically contact each other in many points [20]. These contact sites are not simply a consequence of contiguity but constitute specialized functional areas. Molecular determinants of these contact sites are beginning to be disclosed [21]. A major role of these intracellular "synapses" would be to control the Ca^{2+} flux between the two organelles [20]. Mitochondria behave as a high-capacity, low-affinity transient calcium store. The inward current is mainly dependent on the mitochondrial transmembrane

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potential (mtΔΨ)-driven Ca^{2+} uniport [22]. The mitochondria–ER contact site provides a microdomain where the concentration of Ca^{2+}, released by the opening of the ER Ca^{2+} channel(s), can reach localized high concentrations, thereby enabling the functioning of the mitochondrial uniporter [20]. By this way, the ER may communicate with mitochondria via Ca^{2+} signaling without raising the bulk cytosolic Ca^{2+} level over a physiological threshold.

3. Mitochondrial and ER dysfunction caused by HCV infection

As stated above, HCV infection has been associated with enhanced oxidative stress as well as with alteration of Ca^{2+} homeostasis although their inter-play has scarcely been elucidated. A number of *in vitro* studies reported that overexpression of specific HCV proteins (mainly the core protein) resulted in enhanced production of ROS accompanying other mitochondrial dysfunctions [4,7,23]. Similar results were obtained in cells bearing HCV replicons or infected with cell culture-derived HCV as well as in liver mitochondria derived from HCV transgenic mice [7,24,25]. Some of the effects observed could be recapitulated by incubation of purified recombinant HCV proteins with isolated mitochondria [26,27].

Recently, we investigated the impact of the HCV protein expression on the mitochondrial bioenergetics using a well-characterized inducible cell model system [5,28]. U2-OS human osteosarcoma cells were engineered to inducibly express the entire HCV ORF under the control of a tetracycline-regulated gene expression system. Although not supporting genuine HCV replication, these cells represent a reliable system to study the effect of the viral proteins in the temporal window preceding the replicative phase of the HCV life cycle. By means of this cell model, we provided evidence that the concerted expression of HCV proteins causes enhanced mitochondrial entry of Ca^{2+} [5]. This event anticipated all the other mitochondrial alterations comprising inhibition of the RC complex I, decrease of the mtΔΨ and of the oxidative phosphorylation (OXPHOS) efficiency, generation of ROS ($O_2^{\cdot -}$ and H_2O_2) and reactive nitrogen species (RNS) [5]. All these events were sensitive to treatment with specific inhibitors of the mitochondrial Ca^{2+} uniporter (ruthenium red or RU360). Thus, we concluded that the primary event triggering successive HCV-related cell alterations is an enhanced load of Ca^{2+} into mitochondria.

To prove the contribution of the ER as calcium source we tested the effect of the specific inhibitor of the ER calcium channel(s) dantrolene [29] on the mitochondrial alterations elicited by HCV protein expression in U2-OS. Fig. 1 clearly shows that treatment of induced cells with dantrolene completely prevented (i) mitochondrial Ca^{2+} overload, (ii) ROS production, (iii) dissipation of mtΔΨ, and (iv) inhibition of RC complex I.

In order to define if a specific HCV protein was responsible for the observed mitochondrial alterations we extended the analysis to U2-OS

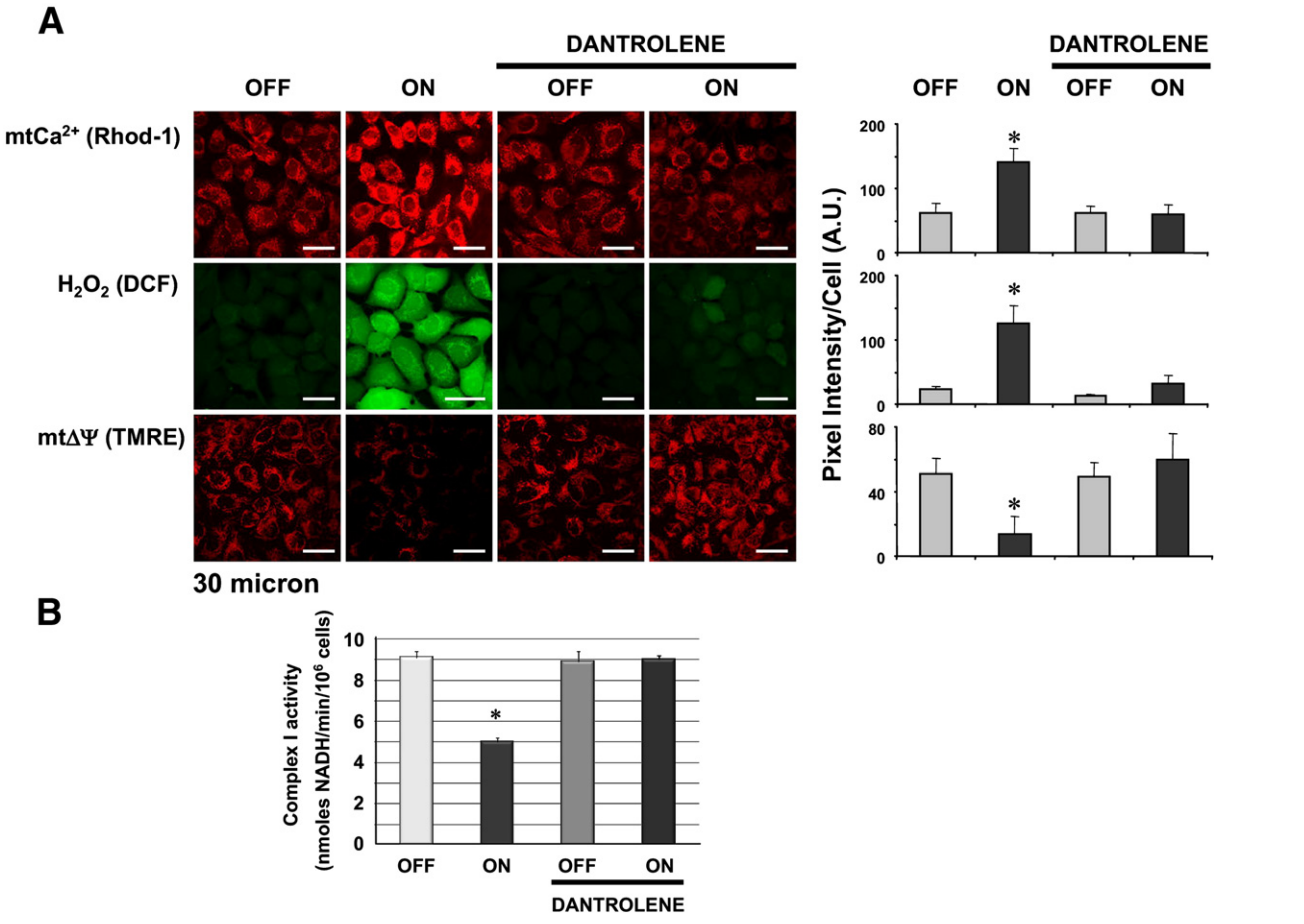

Fig. 1. HCV protein-related mitochondrial dysfunction is linked to Ca^{2+} efflux from the ER. U2-OS cells were engineered to inducibly express the entire HCV polyprotein under the control of a tetracycline-regulated gene expression system [5, 28]. Induction was performed for 48 h. When indicated 10 μM of dantrolene was present in the media. (A) Laser scanning confocal microscopy analysis (LSCM) of intramitochondrial Ca^{2+}, hydrogen peroxide, mtΔΨ monitored by the fluorescence probes rhod1, DCF, TMRE respectively. Left side: imaging of the specific probes-linked fluorescence, representative of three different experiments yielding comparable results. Right side: quantitative analysis of the fluorescence intensity signal. Digitized images were processed by ImageJ, 10 different fields were randomly selected for each condition and the pixel intensity measured within the outline of each cell (each field containing 15–20 cells). The values are means ± SEM; *, $P<0.001$ *vs.* all the other conditions. No significant difference was measured among all the other paired experimental settings. (B) Effect of dantrolene on the mitochondrial RC complex I. Measurement of the NADH dehydrogenase activity was performed spectrophotometrically on cell lysates as in [5]. The values are means ± SEM of 3 different experiments for each experimental setting; *, $P<0.0005$ *vs.* all the other conditions. OFF, non-induced cells; ON, induced cells.

cell lines inducibly expressing subgenomic constructs comprising either the HCV structural or the nonstructural proteins alone (Fig. 2). It is shown that the expression of both structural and nonstructural HCV proteins elicited comparable effects, i.e., enhanced mitochondrial Ca^{2+} load and H_2O_2 generation and dissipation of mtΔΨ. All these alterations were reversed by treatment with ruthenium red. Moreover, both structural and nonstructural HCV proteins caused a similar substantial inhibition of RC complex I activity also sensitive to ruthenium red treatment. It is worth noting that ruthenium red was efficient even after the full establishment of HCV protein-related alterations. Thus, its action was not simply preventing but rescuing (at least in our experimental setting).

Two possible explanations can be offered to interpret the results above. One would rule out the role of a single specific protein in HCV-induced mitochondrial dysfunction in favor of a more general effect linked to overexpression of proteins and their ER-localized translation. Overloading of protein at the ER membrane proved to cause the UPR, which is an adaptive reaction to optimize folding and post-translational redox modifications of translated products [30]. The UPR is mediated by ER calcium depletion. Consistently HCV-related ER stress and Ca^{2+} release activating signaling pathways have been reported [3,31–33]. An alternative explanation is that one structural and one nonstructural HCV protein might exert a similar redundant effect being sufficient to cause Ca^{2+}-linked mitochondrial dysfunction even when expressed separately.

To exclude that the observed HCV-linked mitochondrial dysfunction was not limited to a specific *in vitro* cell system, we have recently extended our analyses to Huh-7.5 (human hepatocellular carcinoma) cells transfected with an HCV genome able to sustain the complete viral life cycle in tissue culture [34,35]. The insertion of the a GFP-coding gene in frame within the C-terminus of HCV NS5A allowed to track infected Huh-7.5 cell lines [36]. Using this cell model, we tested the level of the mtΔΨ production in HCV-infected cells. Fig. 3 clearly shows that only infected cells harboring HCV displayed a marked decrease of the mtΔΨ. Moreover, analyses of cell subsets showed a correlation between the level of infection (monitored by the fluorescence intensity of the NS5A-GFP fusion protein) and the extent of the mtΔΨ. Importantly, treatment of infected Huh-7.5 cell line with either ruthenium red or dantrolene, likewise for HCV-induced U2-OS cells, resulted in a marked ablation of both the mtΔΨ

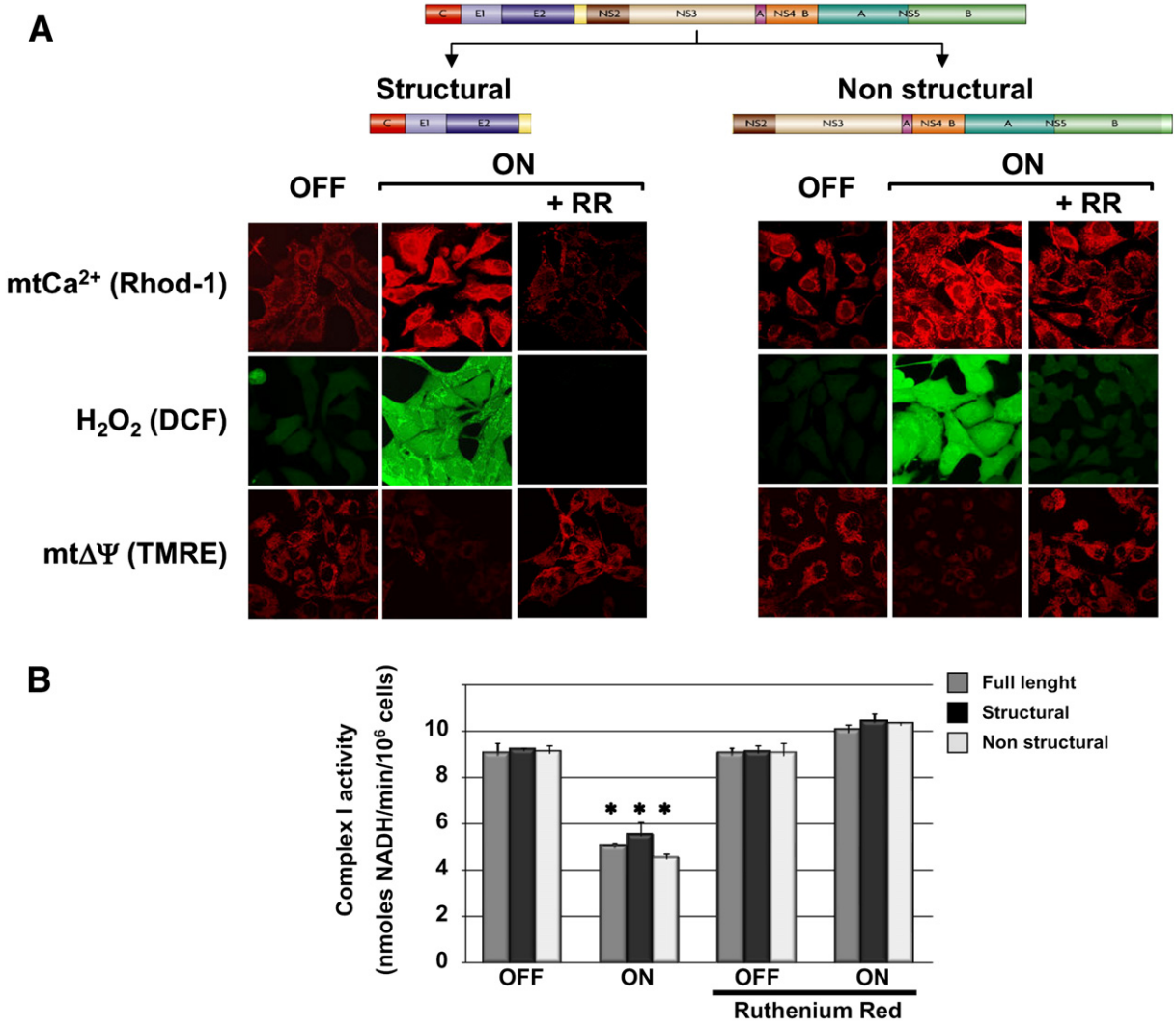

Fig. 2. HCV-linked mitochondrial dysfunction is not elicited by a single specific HCV protein. U2-OS cells were engineered to inducibly express either the HCV structural region as well as the p7 polypeptide (left panel) or the nonstructural proteins 3–5B (right panel) (D. Moradpour, unpublished data). HCV proteins were induced for 48 h. (A) LSCM analysis of intramitochondrial Ca^{2+}, hydrogen peroxide, mtDY monitored by the fluorescence probes rhod1, DCF, TMRE respectively. The effect of 5 μM ruthenium red treatment (added in the last 12 h of induction) is also shown. OFF, non-induced cells; ON, induced cells. The images displayed in the two composite panels are representative of three different cell preparations for each condition giving comparable results. (B) Effect of structural and nonstructural HCV protein expression on NADH dehydrogenase activity of RC complex I. The enzymatic assay was carried out spectrophotometrically on cell lysates as in [5] and the values reported were corrected for the rotenone-sensitivity activity. The effect of ruthenium red treatment on non-induced and induced cells is also shown. Average of 3–4 different assays, for each condition, ± SEM.

collapse and ROS production (authors' unpublished results). Collectively these observations highlight that the HCV-linked mitochondrial dysfunction is not confined to a specific cell type or to the modalities of the viral protein expression but rather is a general hallmark of HCV-cell host interaction.

4. HCV-related cell bioenergetic alterations

An important point to be considered concerns the biological consequences of the HCV-induced redox unbalance in the infected cell. Studies reported that HCV protein expression elicits either stimulation [37–39] or inhibition [40–44] of cell death. In our *in vitro* system, the inducible expression of the HCV proteins did not result in cell distress (i.e. reduced cell viability) when the induction was performed for 48 h [5]. However, when the induction protocol was maintained for periods longer than 48 h a cell density-dependent decline in cell growth rates was observed. This would indicate that the production of ROS was kept initially below the cytotoxic level but that a progressive chronic nitro-oxidative insult resulted in impairment of the cell cycle and/or in cell death. This obvious conclusion may turn out, however, to be an over-simplification not accounting for the potential adaptive response of the cell. Surprisingly, ATP measurements showed higher concentrations in cells induced for 5 days than in non-induced cells [unpublished data]. Thus, in spite of the HCV-linked mitochondrial dysfunction the induced cell still preserved a full bioenergetic competence. However, when cells were grown in a galactose-based medium HCV induction resulted in severe impairment of ATP production and pronounced cell-death [5]. Taken together, these results indicate that when the cell metabolism is not forced to rely exclusively on oxidative phosphorylation, HCV proteins somehow induce an adaptive response conferring pro-survival to the infected cells.

To further explore the basis for this observation, we investigated whether there was an up-regulation of the glycolytic pathway. Indeed a three-fold increase of lactate release in the culture medium was measured in HCV-induced U2-OS cells [unpublished data]. All the glycolytic enzymes as well as the glucose transporter(s) are under transcriptional control of the hypoxia induced factor (HIF) [45,46]. Activation of HIF depends on the hypoxia-dependent stabilization of subunit HIF-1α, which under normoxic conditions is rapidly proteolysed following O_2-dependent post-translational modification [47]. However factors in addition to hypoxia have been described to stabilize HIF-1α [48].

Interestingly a recent study reported that HCV-infected Huh-7 cells stabilize HIF-1α under normal oxygen tension [49]. Moreover, the HCV-related HIF-1α stabilization was sensitive to treatment of the

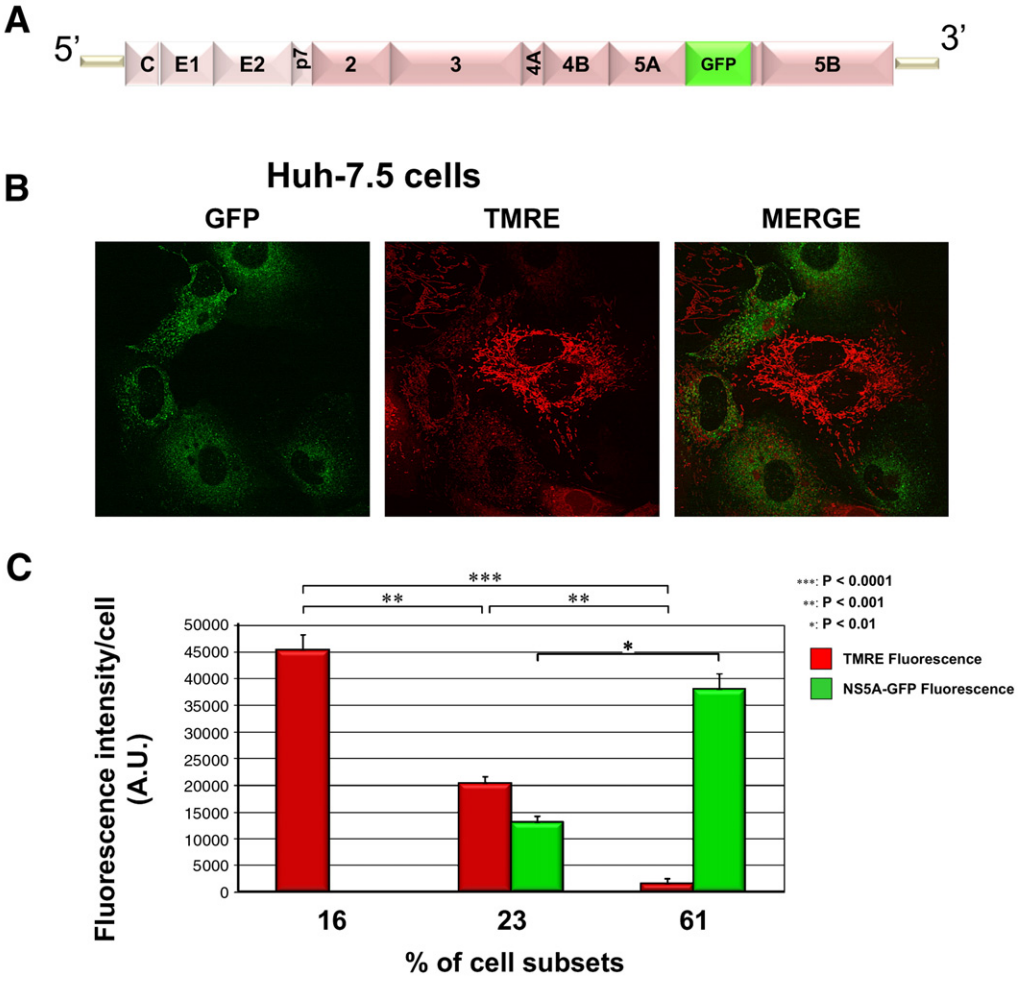

Fig. 3. Detection of mtΔΨ in infected HCV-replicating Huh 7.5 cells. A fully replication-competent HCV genome harbouring a GFP insertion in NS5A (A) was micro-electroporated into Huh-7.5 cells (Digital Bio Technology) [36]. (B) LSCM analysis of mtΔΨ evaluated by the TMRE fluorescent probe. The electroporated Huh-7.5 cell sample was incubated with 2 μM TMRE as in [5] and sequentially scanned to detect first the GFP-related green fluorescence and then the TMRE-related red fluorescence in the same optical field. The image shown is representative of the results obtained from 20 randomly selected fields. (B) Quantitative analysis from single cell-measurements of the green/red fluorescence intensity. Acquisition, storage and analysis of data were performed with LaserSharp and LaserPix software from Biorad. The values shown are means ± SEM from cells selected on the basis of null, intermediate and high GFP-fluorescence. The overall number of cells analysed was 250 (from two different experiments) and the subsets expressed as percentage of the entire cell population. Two-tailed Student *t*-test was applied to estimate the indicated *P* value.

infected cells with calcium chelating agents or with antioxidants [49]. We have recently extended these observations confirming an HCV-dependent activation of HIF-1α and an up-regulation of HIF-controlled gene expression both in cell cultures and in hepatitis C affected patients [M. Ripoli et al., manuscript in preparation]. Of note, in addition to providing a metabolic adaptive response to hypoxia, HIF controls the transcription of a wide range of factors involved in angiogenesis, cell proliferation, survival, apoptosis and many other cell pathways [50]. Therefore, its activation has been implicated in the onset, progression and diffusion of solid tumours [51].

5. Sequence of HCV protein-induced mitochondrial alterations

Fig. 4 illustrates a possible sequence of events following HCV infection that combine evidence provided by our group with others present in literature and offers a mechanistic working model amenable to further validation. It is proposed that although the bulk of HCV proteins accumulate at the ER and ER-derived modified membranes [2], a portion that specifically localizes to ER–mitochondria contact sites is responsible for the observed HCV-linked mitochondrial alterations. The priming event is the release of Ca^{2+} from ER stores by opening of the Ca^{2+} channel(s). The mechanism is not fully understood but a direct interaction or an ER stress-mediated indirect effect can both be considered and are not mutually exclusive [31–33]. The activated outward flux of Ca^{2+} from the ER enhances its local concentration at ER–mitochondria contact sites without increasing the bulk cytoplasmic Ca^{2+} concentration [20]. The entry of Ca^{2+} into mitochondria is mediated by the voltage-dependent anion channel (VDAC)/porin of the OMM and by the ΔΨ-driven Ca^{2+} uniporter of the IMM [22,52–54]. Direct interaction of HCV proteins with the mitochondrial transport system(s) cannot be excluded [24,25]. Intermembrane transfer of proteins from the ER to the OMM is shown as a possible mechanism to account for the reported localization of HCV proteins on mitochondria [14]. Although the viral proteins confine to the OMM, effects on IMM constituents might take place via intermembrane protein–protein interaction likely occurring at the OMM–IMM contact sites. Core, NS3/4A and NS5A might be possible specific candidates in case of a direct interaction with mitochondrial components [14,18,19,24–27]. The main mitochondrial exit route of Ca^{2+} is mediated by the Na^+/Ca^{2+} exchanger and by transient opening (flickering) of the mitochondrial permeability transition pore (MPTP). [22,53]. Re-uptake of Ca^{2+} by the ER is dependent on the Ca^{2+} ATPase.

Enhanced recycling of Ca^{2+} is expected to decrease the mtΔΨ and thereby the efficiency of ATP synthesis [22,53]. However, this effect is compensated by the Ca^{2+}-mediated activation of the Krebs' cycle dehydrogenases, which enhancing the reducing substrate availability for the RC, up-regulates the OXPHOS whereby leading to a prompt adaptive response to the energy-requiring stressing condition [54]. This mechanism may be beneficial, if time controlled, to overcome transient bioenergetic impairment but becomes harmful when the insult persists for extended periods as in the case of the chronic viral infection [54]. High levels of mitochondrial Ca^{2+} may cause changes in the redox balance by a number of described mechanisms that are not mutually exclusive. Consistent with our observations it is worth-mentioning that mitochondrial Ca^{2+} overload was shown to displace cytochrome *c* from the IMM-cardiolipin slowing down the flux of

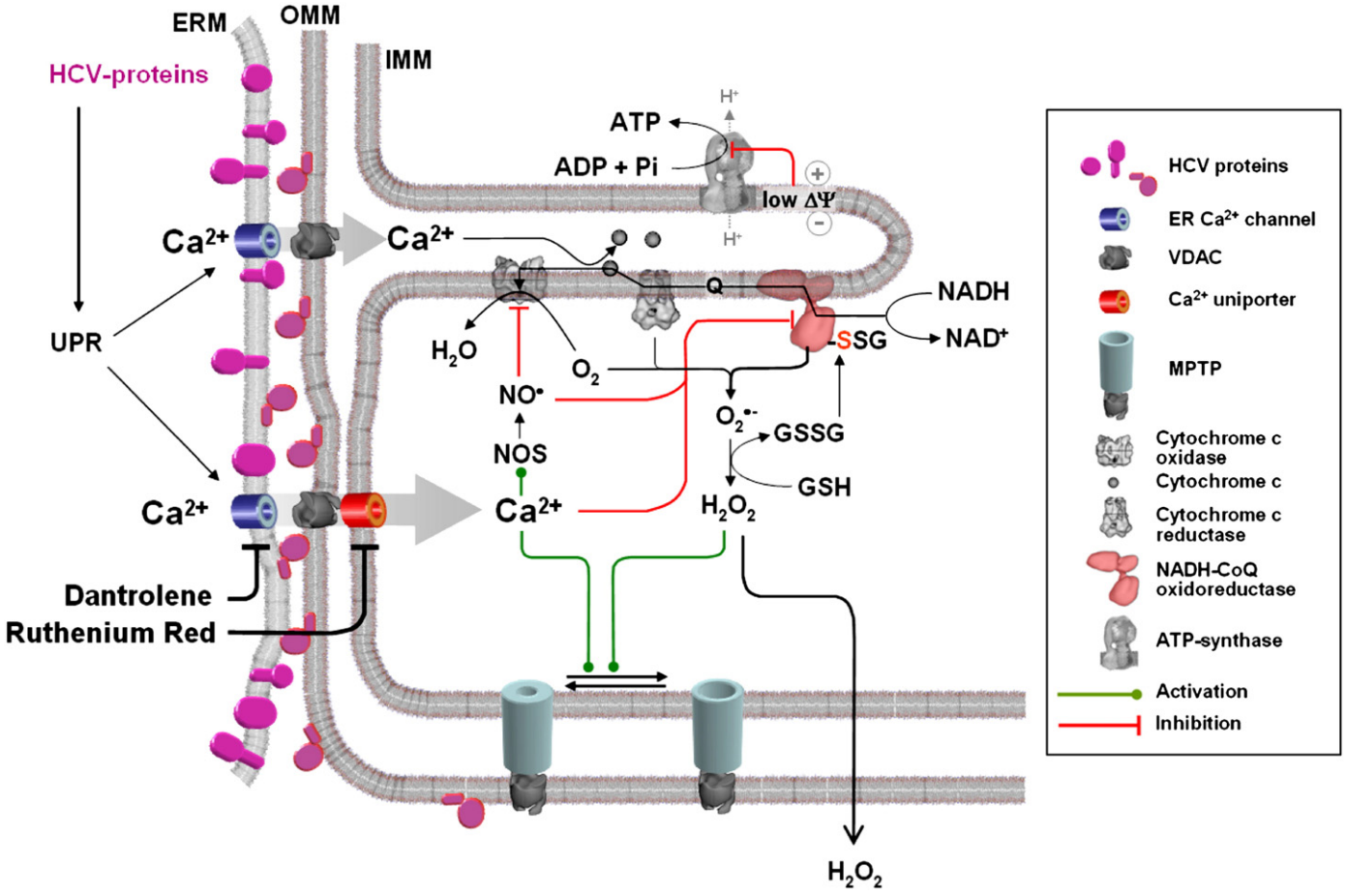

Fig. 4. Sequence of HCV protein-induced mitochondrial alterations. An ER-mitochondria contact site is schematically drawn. HCV proteins are shown in purple on the ER and OMM. The different shapes reflect their level of integration with the phospholipids bilayer. These are supposed to transfer by lateral diffusion from ER to the OMM. Direct interaction of HCV protein with both the ER- and OMM-located Ca^{2+} transporters elicits release of Ca^{2+} from the ER and/or uptake of Ca^{2+} within the mitochondria. Herein the enhanced Ca^{2+} steady-state level ensues a cascade of events leading to dysfunction of the OXPHOS system and progressive change in the redox state. See text for description of the model.

electrons between the cytochrome *c* reductase and the cytochrome *c* oxidase [53,55]. In addition, Ca^{2+} activates the mitochondrial isoform of a nitric oxide synthase, thereby releasing $NO^{\cdot}$, which is an inhibitor of cytochrome *c* oxidase [56]. The synergistic combination of these two events are expected to cause accumulation of reducing equivalents upstream of cytochrome *c* oxidase, resulting in electron leakage to O_2 with formation of $O_2^{\cdot -}$ over the basal level [57]. Moreover, NO and Ca^{2+} can directly inhibit the RC complex I, enhancing ROS production by this complex [58,59]. In order to counterbalance this increase of ROS production the intramitochondrial redox buffer (mainly GSH) becomes progressively depleted. Re-reduction of GSSG by glutathione reductase requires NADPH, which is largely provided by the mitochondrial NADH/NADPH trans-hydrogenase activity [60]. However, this is an IMM enzyme catalyzing a $\Delta\Psi$-driven reduction of $NADP^+$. Thus, it is tempting to speculate that the reduced $mt\Delta\Psi$ observed in mitochondria of HCV-infected cells would limit the trans-hydrogenase activity, leading in turn to accumulation of oxidised glutathione (GSSG). GSSG can form mixed disulphide bonds with protein cysteine thiols and the RC complex I was shown to be susceptible to reversible glutathionylation, which causes partial inhibition of its activity and enhanced generation of $O_2^{\cdot -}$ [61]. Moreover, ROS has been shown to affect the ER Ca^{2+} channels [53]. These last processes would therefore fuel a positive feedback mechanism. Their occurrence in cell models of HCV-infection warrants further investigation and in this respect experimental work is in progress in our lab.

6. A pathogenic model for HCV-related liver diseases

A major point to be discussed concerns the biological effects, in terms of cell fate, that the oxidative unbalance causes in HCV infected cells. This issue has to be considered taking in account the strategy of interaction between the virus and the infected host cell. On the one hand, if the infected cell is not able to clear HCV infection by immune or other (e.g., autophagy) protective mechanisms it might be convenient to provoke cell death in order to limit virus dissemination in the organism. On the other hand, from the viewpoint of the virus it is convenient to interfere with the auto-disruption of the host cell to fully exploit it as energy and matter source needed to nourish replication and virus assembly. Some viruses, in the late stage of their life cycle, favour the death of the host cell to promote dissemination of the infection. However, this does not apply to HCV, which is non-cytolytic.

In the scheme shown in Fig. 5 a pathogenic model is presented whereby the level of the redox unbalance is proposed to modulate the variable pathological outcomes of the HCV infection. Indeed, depending on compartmentalization, amount, frequency of production, and specific chemical species, ROS proved to result in signaling for a broad

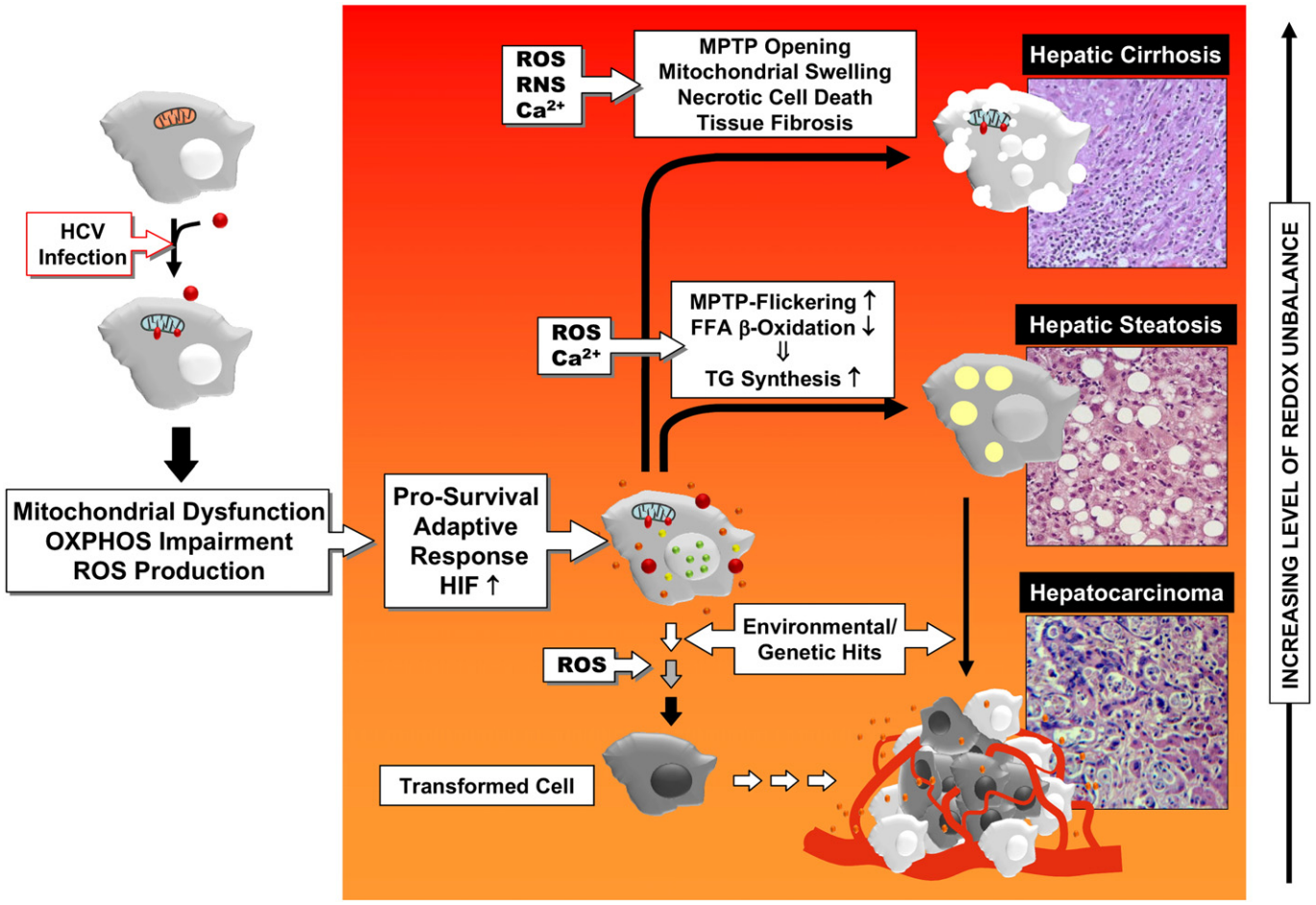

Fig. 5. Proposed pathogenic role of mitochondria in the development of HCV-related diseases. Consequently to the HCV-induced dysfunction of the mitochondrial OXPHOS system an adaptive bioenergetic response of the cell is ensued. In this context the pathological outcomes of the HCV infection are suggested to depend on the level of the oxidative alteration. i) Low ROS level re-enforces a pro-survival and proliferative phenotype by redox signalling. If additional hits (mutagenic) accumulate over the time this may result in clonogenic expansion leading to hepatocarcinoma. ii) Intermediate levels of ROS and Ca^{2+} enhances the closed to open transition of the MPTP causing depletion of low-molecular weight metabolites needed to import and catabolize long chain acyl-CoA by β-oxidation thereby leading to their cytoplasmic accumulation and conversion in triglycerides. This would lead to steatosis. iii) High intramitochondrial concentrations of Ca^{2+} and ROS induces permanent opening of the MPTP causing osmotic swelling and rupture of the OMM. The consequent release of cytochrome *c* and other pro-apoptotic factors triggers the caspase-cascade. Depending on the intracellular ATP level, this would lead to apoptosis or necrosis activating, in the last case, tissue fibrosis. See text for further explanation.

array of biological effects from cell survival to suicide [62–64]. Intriguingly, contradictory evidence has been reported concerning the effect of HCV protein expression on cell growth [37–44].

It is proposed that low production of ROS is not harmful to the cell but instead promotes pro-survival adaptation of HCV infected cells *via* redox signalling-mediated mechanism [62,63]. This might be further favoured by described HCV-related activation of antiapoptotic pathways (i.e. HIF, NF-kB, JNK, STAT3) [26,49]. However, the proliferative advantage of HCV-infected cells under low ROS generation regime can lead to accumulation of mutagenic hits resulting in carcinogenic priming of the host cell and ultimately in HCC development [51,64].

At intermediate level of oxidative insult (and Ca^{2+} deregulation) a possible link between mitochondrial dysfunction and altered lipid metabolism (hallmarking HCV infection [65]) may establish. HCV-linked cirrhotic progression relies, at the cell biochemical level, on increased triglyceride synthesis, which likely occurs because of defective free fatty acid (FFA) oxidation. Utilization of FFA takes place mainly within the mitochondrial matrix by mean of the β-oxidation pathway. The entry of activated long-chain FFA as acyl-CoA into mitochondria requires conversion to acyl-carnitine by the carnitine acyl-CoA transferase (CAT1-isoform) which is located in the mitochondrial inter-membrane space [66]. A carnitine/acyl-carnitine translocase allows acyl-carnitine to enter the mitochondria in exchange with carnitine. The compartmentalization of this process allows prompt replenishing of free carnitine in the inter-membrane space. As opening of the MPTP allows rapid equilibration of solutes of molecular weight up to 1500 Da [67,68] it is conceivable to hypothesize that enhanced flickering of the MPTP might cause low molecular weight molecules like carnitine (or CoA (M.W. 162 and 767 Da respectively)) to diffuse and dilute in the cytoplasm. This would cause a loss of the micro-environmental control of the conversion of acyl-CoA into acyl-carnitine. Thus, accumulating acyl-CoA molecules are forced toward triglyceride synthesis, with progressive formation of lipid droplets leading to steatosis in HCV-infected hepatocytes. Deregulation of metabolism-controlling master transcription factors, like the peroxisome proliferators activating receptor alpha (PPAR-α) [69], would further exacerbate the lipogenic process.

High concentrations of mitochondrial Ca^{2+}, ROS ($O_2^{\cdot -}$, H_2O_2) and RNS ($NO^{\cdot}$, $ONOO^-$) are all powerful triggers of the mitochondrial MPTP permanent opening [70]. The resulting osmotic swelling (caused by the large intramitochondrial oncotic pressure) causes rupture of the OMM and release of cytochrome *c* and other pro-apoptotic factors, which initiates a practically irreversible step in the apoptotic program [10]. However, depending on the intracellular ATP level the apoptotic program can diverge toward necrotic cell death [71]. Thus, high ROS production regimes may overwhelm the pro-survival setting resulting in HCV-related inflamatory fibrogenesis.

The different pathogenic outcomes related to the variable level of the HCV-linked oxidative insult may depend on differences in the cellular virus load or viral protein expression and/or on age-dependent factors of the host.

Acknowledgments

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