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Original Contribution

Mitochondria are targets for peroxisome-derived oxidative stress in cultured mammalian cells

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ABSTRACT

Many cellular processes are driven by spatially and temporally regulated redox-dependent signaling events. Although mounting evidence indicates that organelles such as the endoplasmic reticulum and mitochondria can function as signaling platforms for oxidative stress-regulated pathways, little is known about the role of peroxisomes in these processes. In this study, we employ targeted variants of the genetically encoded photosensitizer KillerRed to gain a better insight into the interplay between peroxisomes and cellular oxidative stress. We show that the phototoxic effects of peroxisomal KillerRed induce mitochondria-mediated cell death and that this process can be counteracted by targeted overexpression of a select set of antioxidant enzymes, including peroxisomal glutathione *S*-transferase Kappa 1, superoxide dismutase 1, and mitochondrial catalase. We also present evidence that peroxisomal disease cell lines deficient in plasmalogen biosynthesis or peroxisome assembly are more sensitive to KillerRed-induced oxidative stress than control cells. Collectively, these findings confirm and extend previous observations suggesting that disturbances in peroxisomal redox control and metabolism can sensitize cells to oxidative stress. In addition, they lend strong support to the ideas that peroxisomes and mitochondria share a redox-sensitive relationship and that the redox communication between these organelles is mediated not only by diffusion of reactive oxygen species from one compartment to the other. Finally, these findings indicate that mitochondria may act as dynamic receivers, integrators, and transmitters of peroxisome-derived mediators of oxidative stress, and this may have profound implications for our views on cellular aging and age-related diseases.

Introduction

Peroxisomes are highly dynamic cell organelles that play key roles in multiple metabolic pathways [1]. In mammals, these include—among others—the breakdown of various carboxylates via α- and β-oxidation and the biosynthesis of docosahexaenoic acid and ether phospholipids [2,3]. Many of the enzymes involved in these processes produce reactive oxygen species (ROS)[1] as part of their normal catalytic activity [4]. Mammalian peroxisomes also contain various ROS-detoxifying enzymes, including catalase (EC 1.11.1.6, CAT), Cu/Zn-superoxide dismutase 1 (EC 1.15.1.1, SOD1), glutathione *S*-transferase Kappa 1 (EC. 2.5.1.18, GSTK1), epoxide hydrolase 2 (EC 3.3.2.10), and peroxiredoxin 5 (EC 1.11.1.15) [5]. The importance of peroxisomes for human health and development is best illustrated by the existence of severe inherited metabolic diseases (e.g., Zellweger syndrome and X-linked adrenoleukodystrophy) that are caused by a partial or complete dysfunction of the organelle [6,7]. In addition, there is growing evidence for the involvement of peroxisomes in the etiology and progression of aging and age-related diseases [8]. This may not be so surprising given that changes in peroxisomal metabolism have been suggested to orchestrate developmental decisions (e.g., cell fate) by modulating the cellular composition and concentration of specific lipids and (redox-derived) signaling mediators [9,10]. Unfortunately, little is known about the identity of these signaling pathways and how peroxisomes are integrated into subcellular communication networks [11].

Abbreviations: ALS, amyotrophic lateral sclerosis; c, cytosolic; CAT, catalase; ER, endoplasmic reticulum; GSTK1, glutathione *S*-transferase Kappa 1; HuF, human fibroblast; KR, KillerRed; LA, α-lipoic acid; MEF, mouse embryonic fibroblast; mt, mitochondrial; NAC, *N*-acetylcysteine; PARP, poly(ADP-ribose) polymerase; po, peroxisomal; RIPK1, receptor-interacting protein kinase 1; ROS, reactive oxygen species; SOD1, superoxide dismutase 1

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To fulfill their functions, peroxisomes physically and functionally interact with other cell organelles, including mitochondria, the endoplasmic reticulum (ER), and lipid droplets [1,12]. For example, it is well established that, in mammals, peroxisomes and mitochondria are metabolically linked [13], cooperate in antiviral signaling and defense [14], and share key components of their division machinery [15]. We and others recently found that a disturbance in peroxisomal metabolism triggers signaling/communication events that ultimately result in increased mitochondrial stress [16–18]. In addition, we observed that generating excess ROS inside peroxisomes quickly perturbs the mitochondrial redox balance and leads to excessive mitochondrial fragmentation [16]. The molecular mechanisms underlying these phenomena remain unclear. However, in this context, it is of interest to note that a recent confocal microscopy study has visualized a membrane network, distinct from the ER, which physically connects peroxisomes and mitochondria [19]. These contact sites may facilitate both signaling and the passage of ions and lipids from one compartment to another [20]. On the other hand, it may also be possible that peroxisomal ROS simply diffuse through the peroxisomal membrane via PXMP2, a nonselective pore-forming membrane protein with an upper molecular size limit of 300–600 Da [21]. Finally, it should be mentioned that there is also evidence of a vesicular transport pathway from mitochondria to peroxisomes [22]. However, no data currently exist for such a pathway in the opposite direction.

In this study, we employed a peroxisomal variant of KillerRed (KR), a red fluorescent protein that displays strong phototoxic properties upon green light illumination [23], to gain a better insight into the downstream cellular effects of excess peroxisomal ROS production. In addition, we compared these effects with those of cytosolic and mitochondrial variants of KR. Importantly, previous studies have already shown that targeted variants of KR can be used as powerful tools to study the downstream effects of local ROS production. For example, it has been demonstrated that a membrane-tethered version of this genetically encoded photosensitizer can be used to manipulate the viability and/or function of KR-expressing cells in transgenic zebrafish [24]. Others have shown that mitochondrial KR can be used to robustly initiate parkin-mediated autophagy in a spatially and temporally controlled fashion [25] and to expand our understanding of the role of mitochondrial oxidative stress in cell fate decisions [26,27].

We show here that peroxisomal KR can be used to gain a better insight into factors that may contribute to or influence redox signaling between peroxisomes and mitochondria. Our findings provide strong novel evidence that (i) disturbances in peroxisomal metabolism sensitize cells to KR-induced oxidative stress, (ii) excessive peroxisomal ROS production elicits mitochondria-mediated cell death, and (iii) the redox communication between peroxisomes and mitochondria involves complex signaling pathways. The implications of these findings for how peroxisomes can be integrated into cellular communication networks are discussed.

Materials and methods

DNA manipulations and plasmids

The plasmids encoding nontagged versions of human SOD1 and $SOD1_{G93A}$ were kindly provided by Dr. L. Van Den Bosch (Laboratory for Neurobiology, KU Leuven, Belgium). The plasmid encoding green fluorescent protein (GFP)–Bax was kindly provided by Dr. R. J. Youle (National Institutes of Health, Bethesda, MD, USA). The mammalian expression vectors pEGFP-N1 (Clontech), pKillerRed-dmito (Bio-Connect), and pCR2.1 TOPO (Invitrogen) were commercially obtained. The plasmids encoding CAT, po-KR, mt-KR, c-KR, po-roGFP2, mt-roGFP2, or c-roGFP2 have been described elsewhere [16]. The oligonucleotides used to amplify PCR products are listed in Supplementary Table 1. The plasmid encoding cytochrome *c*–EGFP (pMF1807) was constructed by amplifying the cDNA fragment encoding human cytochrome *c* by PCR (template, human liver cDNA library; primers, HsCytc.fw1 and HsCytc.rv1; nested primers, HsCytc.fwBglII and HsCytc.rvHindIII) and cloning the *Bgl*II/*Hin*dIII-digested PCR product into the *Bgl*II/*Hin*dIII-cut pEGFP-N1 vector. The plasmid encoding nontagged po-CAT (pMF1526), a human catalase variant containing a strong peroxisomal targeting signal, was generated by ligating the PCR-amplified HsCatalase-SKL cDNA fragment (template, pJK18 [28]; primers, HsCatalase.1fwBglII and pBADHisrvNotIPstI; digested with *Bgl*II/*Pst*I) into the *Bgl*II/*Pst*I-cut pEGFP-N1 vector. The construct encoding mt-CAT (pMF1763) was generated by ligating the PCR-amplified HsCatalaseΔKANL cDNA fragment (template, pJK20 [28]; primers, HsCatalase.1fwBglII and pBADHisrevNotIPstI; digested with *Bgl*II/*Not*I) into the *Bgl*II/*Not*I-cut backbone fragment of pKillerRed-dmito. The plasmid encoding mt-SOD1 (pOI19) was created by cloning the PCR-amplified human SOD1 cDNA fragment (template, nontagged HsSOD1 (see above); primers, SOD1fw-BamHI and SOD1revNotI; digested with *Bam*HI/*Not*I) into the *Bam*HI/*Not*I-cut backbone fragment of pKillerRed-dmito. The mammalian expression vector encoding 3xmyc-tagged po-SOD1 (pOI17) was constructed by ligating the PCR-amplified SOD1-SKL cDNA fragment (template, nontagged HsSOD1 (see above); primers, SOD1fwBamHI and SOD1PTS1rvNotI; digested with *Bam*HI/*Not*I) into the *Bam*HI/*Not*I-digested pMP1 plasmid [29]. The plasmid encoding $SOD1_{H46R}$ (pBW2) was constructed by fusion PCR. In a first PCR, two PCR fragments (template, nontagged HsSOD1; primers, SOD1fwBamHI and SOD1H46Rrv (fragment 1) or SOD1H46Rfw and SOD1rvNotIBglII (fragment 2)) were generated. These fragments were fused and used as templates in a second PCR (primers, SOD1fwBamHI and SOD1rvNotIBglII). After digestion with *Bam*HI/*Not*I, the fusion fragment was subcloned into the *Bam*HI/*Not*I-cut backbone fragment of the pEGFP-N1 vector. To generate the plasmid encoding HsGSTK1-roGFP2-PTS1 (pKM1558), the PCR-amplified cDNA fragment encoding HsGSTK1 (template, human cDNA pool; primers, oli_1015 and oli_1828) was subcloned into pCR2.1 via TOPO-TA cloning, and the open reading frame was subsequently excised with *Bgl*II and *Hin*dIII and cloned into the *Bgl*II/*Hin*dIII-cut plasmid encoding peroxisomal roGFP2. The plasmid encoding $HsGSTK1_{S16A}$-roGFP2-PTS1 (pKM1620) was generated by in vitro mutagenesis of plasmid pKM1558 using the Quikchange in vitro mutagenesis kit (Agilent) in combination with the oligonucleotides oli_1872 and oli_1873. The construct encoding mitochondrial HsGSTK1-roGFP2 (pKM1691) was generated via a three-point ligation of the following fragments: (i) the *Nde*I/*Bsr*GI-cut backbone fragment of pEGFP-N1; (ii) the *Nde*I/*Bgl*II-cut fragment of pKM1132, a pEGFP-N1 derivative encoding the 32-amino-acid mitochondrial targeting signal of human ornithine transcarbamoylase; and (iii) the *Bgl*II/*Bsr*GI-cut fragment of pKM1620, which encodes HsGSTK1-roGFP2. All plasmids were verified by DNA sequencing (LGC Genomics).

Antibodies

The mouse monoclonal antibody against β-actin (Sigma), the rabbit polyclonal antibodies against cleaved caspase-3 (Cell Signaling) and poly(ADP-ribose) polymerase (PARP; Cell Signaling), and the goat anti-mouse and anti-rabbit IgG′s coupled to alkaline phosphatase (Sigma) were commercially obtained. Note that the anti-PARP antibody recognizes both the full-length protein (~116 kDa) and the large fragment of PARP (~85 kDa) resulting from caspase cleavage.

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