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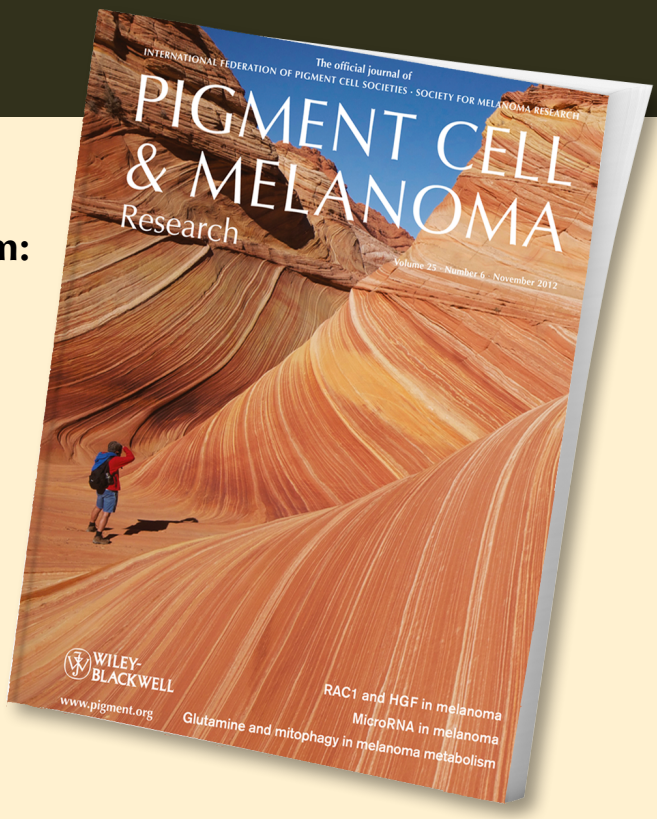

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María S. Soengas

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María S. Soengas

Melanoma Laboratory, Molecular Pathology Programme, Centro Nacional de Investigaciones Oncolœgicas (Spanish National Cancer Research Centre), Madrid, Spain

CORRESPONDENCE María S. Soengas, e-mail: msoengas@cnio.es

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Summary

Proteins and pathways that control cell fate are placed under intense scrutiny. The same tight regulation applies to essential organelles that can both sustain cell survival or promote self-degradation programs. Mitochondria are perhaps the prime example of cellular machineries with split functions (personalities). As a main source of ATP, mitochondria represent the main powerhouse of eukaryotic cells. However, mitochondrial respiration has the hidden complication of the production of potentially harmful reactive oxygen species (ROS). Moreover, mitochondria holds an armamentarium of stress-response factors, which depending on the context, may lead to pro-inflammatory signals, and to various forms of cell death, ranging from apoptosis to necrosis. A main clearance mechanism to eliminate superfluous, damaged or hyperactive mitochondria is selective mitophagy. Mitophagy, in fact, is emerging as a key quality-control mechanism in cancer cells. Specifically, malignant transformation has been found to induce marked changes in mitochondrial dynamics and structure. Moreover, a key hallmark of tumor progression is metabolic reprogramming, which further deregulates ROS content and renders cells more susceptible to mitochondrial perturbations. Despite its increasing relevance in cancer biology, the field of mitophagy remains virtually unexplored in melanoma. However, given unique antioxidant mechanisms in melanocytic cells (e.g., linked to melanin) and the idiosyncratic interplay between ROS and hypoxia (both mitophagy inducers) in melanoma, this tumor type represents an ideal scenario for physiological studies of mitochondrial turnover. This perspective summarizes proof of concept for in-depth basic and translational studies of mitophagy in melanoma. Particular emphasis is dedicated to new opportunities for gene discovery and drug design in this still aggressive disease.

Introduction

Few fields in the cancer arena can rival melanoma in the rise from a 'black box' (Chin et al., 1998) to a paradigm of molecularly targeted therapy (Flaherty et al., 2012) within a bit more than a decade (Tsao et al., 2012). Progress regarding the molecular mechanisms underlying melanoma development (and suppression) is spectacular. The last counts of mutations identified in this disease are impressive, with an average of over 78000 somatic base substitutions in a single genome (Berger et al., 2012; Dutton-Regester and Hayward, 2012; Hodis et al., 2012; Krauthammer et al., 2012; Pleasance et al., 2010; Wei et al., 2012). The 5764 somatic mutations reported in prostate cancer using similar deep sequencing methods look pale in comparison (Barbieri et al., 2012). We know that all of the famed eleven cancer hallmarks (Hanahan and Weinberg, 2011) are invariably deregulated in melanoma. And yet, we are also aware that the path ahead toward integrating this vast information and translating it into effective treatments is likely to be a bumpy road. Perhaps one of the most illustrating examples of the

complexity of signaling cascades in melanoma tumors are the multiple feed-back, feed-forward and crossover regulatory loops that impinge on the BRAF/MAPK pathway and its recently developed inhibitors (Dummer and Flaherty, 2012; Poulikakos and Rosen, 2011). In the face of this vast information, is there any fundamental process bearing therapeutic relevance that has been missed? Each melanoma expert may have his/her favorite candidate(s). Here, I will focus on selective degradation of mitochondria (mitophagy). This is a key determinant of cell homeostasis, which remains virtually unaddressed in melanoma. The intent of this perspective is not to provide an in-depth review on mitophagy, as excellent summaries on the topic can already be found in the literature (Ashrafi and Schwarz, 2012; Green et al., 2011; Nunnari and Suomalainen, 2012; Wang and Klionsky, 2011; Westermann, 2010; Youle and Narendra, 2010). Instead, I will present the rationale as to why delving into mitochondrial biogenesis and targeted degradation may open new areas of research in melanoma. In particular, the interplay between reactive oxygen species (ROS), mitochondrial membrane depolarization and cell survival holds great potential in the context of melanoma development and resistance to therapy.

Mitochondria, a rheostat of cell viability

Few cellular compartments have received as much attention in basic and translational research as mitochondria [see (Dang, 2012; Fulda et al., 2010; Nunnari and Suomalainen, 2012) for reviews]. These organelles sit at a central place in the Hall of Fame of cell metabolism since 1930s, when Hans A. Krebs (later a Nobel laureate) published his seminal studies on the tricarboxylic acid cycle (Krebs and Johnson, 1937). In the cancer field, Krebs' influence is shared with his mentor Otto H. Warburg, also holder of a Nobel prize, and father of the now well-accepted contribution of aerobic glycolysis to tumor development [reviewed in (Dang, 2012; Koppenol et al., 2011)]. Intriguingly, ATP production by oxidative phosphorylation at the mitochondria is concomitant to the production of potentially harmful ROS. One to two percent of the oxygen consumed during basal mitochondrial respiration is converted to superoxide and then to hydrogen peroxide (Zorov et al., 2006). ROS may be up to tenfold higher in damaged mitochondria (Gottlieb and Carreira, 2010), for example, as result of hypoxia, nutrient deprivation or treatment with chemotherapeutic agents (Nunnari and Suomalainen, 2012).

Depending on the stimuli and the environmental conditions, excessive ROS may result in different scenarios. These range from inflammation to programmed forms of cell death by apoptosis or necrosis (Fulda et al., 2010). A brief schematic representation of the main signaling cascades involved in mitophagy is depicted in Figure 1 (additional information can be found in excellent recent reviews (Green et al., 2011; Wang and Klionsky, 2011; Weidberg et al., 2011). In short, ROS can promote the activation of pro-inflammatory signals by NF-κB, the inflammasome, and/or pattern recognition receptors that mediate innate immunity programs. On the other hand, ROS and other death inducing signals driven for example by oncogenic stress, low oxygen or a variety of chemotherapeutic agents may lead to the activation of pro-apoptotic members of the Bcl-2 family, such as BAX or BAK, which in turn can promote the loss of mitochondrial membrane potential. Mitochondrial membrane depolarization results in the release of cytochrome c and other death inducer factors such as AIF or SMAC, for subsequent activation of apoptotic caspases. Under situations of severe stress, cells may burst in necrotic programs (see schematic in Figure 1). Therefore and considering key role of mitochondria determining cell fate, this organelle is under tight scrutiny.

Molecular mechanisms of mitophagy

Mitochondria was found engulfed within double membrane autophagosomes short after the process of lysosomal-associated autophagy was discovered [reviewed in (Wang and Klionsky, 2011)]. Therefore, for a time, it was considered that mitochondrial removal was part of a global clearance of cellular organelles by the process of macroautophagy. While functional mitochondria can be subject of unspecific autophagy (Yang and Klionsky, 2010), it is becoming evident that quality-control processes exist in cells to selectively remove those mitochondria with defective membrane potential (Wang and Klionsky, 2011). Selective clearance of dysfunctional mitochondria (mitophagy) limits the potential harmful production of ROS and pro-apoptotic inducers (Lemasters, 2005). For example, a drastic depletion of mitochondria – a purge – might be useful to eliminate mitochondrial DNA copies that contain enough mutations to interfere with function (Gomes and Scorrano, 2012). Moreover, degraded components of mitochondria (amino acids, lipids, and membranes) may be reused or exploited to sustain cell viability, particularly under stress conditions (Ashrafi and Schwarz, 2012). Emphasizing its roles in the maintenance of proper cell homeostasis, defective mitophagy has been associated with Parkinson's disease (Kim and Sack, 2012) and a variety of age-associated pathologies (Green et al., 2011).

As indicated before, mitophagy is an unchartered territory in the melanoma field. In fact, the mechanisms underlying mitochondrial degradation in other tumor types are just being defined. To follow is a simplified summary of key mitophagy events (Figure 1) that have been reported in various cell types [see (Green et al., 2011; Wang and Klionsky, 2011) for recent reviews].

Mitochondrial fission and membrane depolarization

Mitochondria are highly dynamic organelles which can undergo successive rounds of fusion and fission as part of a quality-control process (Westermann, 2010). In partic-

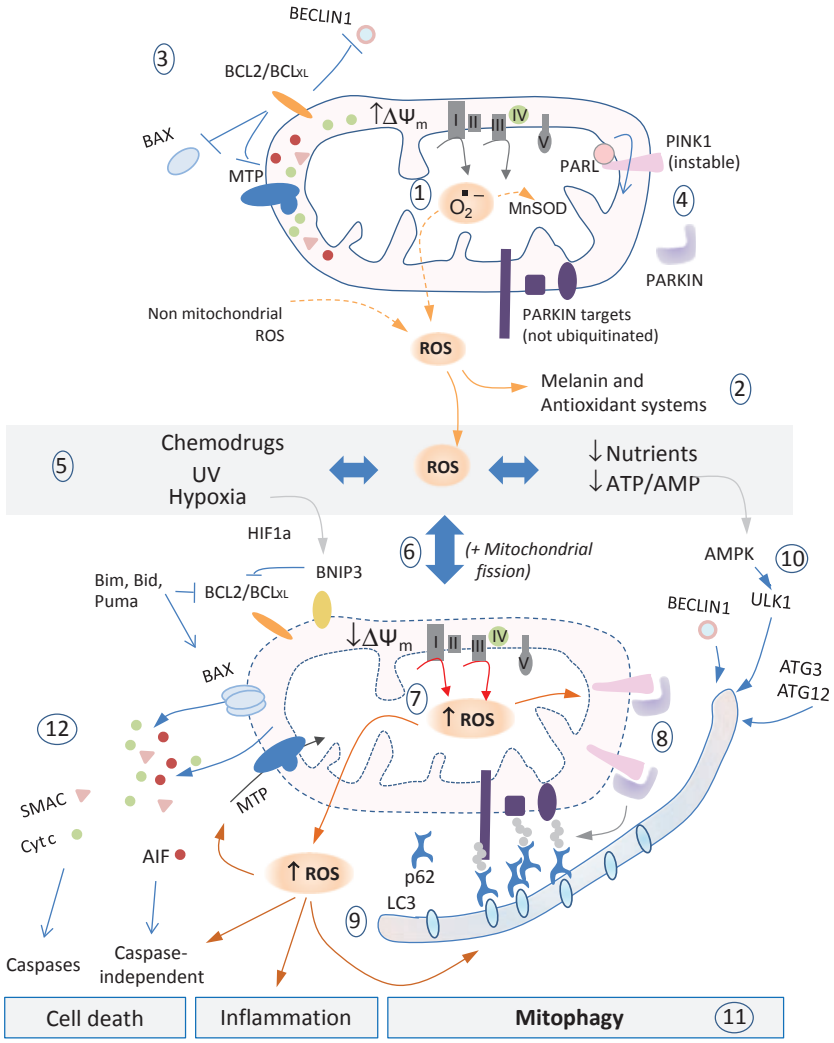

Figure 1. Schematic representation of mitophagy in the context of stress-response programs that deregulate mitochondrial physiology. In basal conditions, moderate reactive oxygen species (ROS) production (1) is efficiently counteracted by mitochondrial and cytosolic antioxidants, including melanin (2). In addition, inhibitory members of the Bcl-2 family (i.e., BCL2 and BCL-xL) block the activation of autophagy via BECLIN1. These Bcl-2 proteins help also maintain the mitochondrial membrane potential ($\Delta\Psi_m$) by restricting pro-apoptotic roles of BAX and proteins that control the mitochondrial permeability transition, MTP (3). In addition, PINK1, a main sensor of mitochondrial stress, is cleaved by mitochondrial proteases such as PARL. In the absence of functional PINK1, PARKIN, an E3-ligase, is not efficiently recruited to the mitochondria. Consequently, there is no ubiquitination and degradation of PARKIN substrates and mitochondria maintain their function (4). In contrast, under situations of extrinsic or intrinsic stress (5), the dynamics and the physiology of mitochondria get altered (6), resulting in the depolarization of the $\Delta\Psi_m$ and the production of higher levels of ROS (7). Under these situations, PINK1 degradation is inhibited and PARKIN ubiquitinates a series of mitochondrial (and cytosolic factors) (8). These ubiquitinated proteins can be recognized by factors such as p62, which in turn can bind the autophagosome factor LC3 (9). The precise roles of p62 and the mechanisms leading to the selective engulfment of damaged mitochondria are not clear, but they may involve the autophagy genes ATG3 and ATG12 (10). The formation of the autophagosome is further favored when BECLIN1 is no longer inhibited by BCL2 or BCL-xL. Additional mediators of autophagosome formation particularly in the context of low concentration of nutrients or defective ATP are AMPK and ULK1 (10). The type, duration and intensity of the stimuli, the intracellular levels of ROS, and the environmental conditions may determine whether cells degrade their defective mitochondria and survive, promote inflammatory responses that may affect also surrounding cells, or enter into different forms of cell death (11). Examples of factors that induce the activation of apoptotic caspases or kill cells in a non-apoptotic manner are depicted (12). See text for additional details of the interplay between stress-inducing stimuli (chemotherapeutic agents, UV, hypoxia, and reduced energetic load) and the outcome of ROS-driven signaling cascades.

ular, healthy mitochondria can be organized in large tubular structures, particularly at the G1-S transition, where at least in some cell types, they can be found as hyperpolarized syncytia (Mitra et al., 2009). These fused mitochondria are too large to be included in the usually small (1 μm) autophagosomes and are thus protected from degradation. For mitophagy to occur, damaged or dysfunctional mitochondrial compartments are usually first fragmented (by fission mechanisms) to form smaller structures (Apostolova et al., 2011). Nevertheless, while

fission is necessary, the key initiator of mitophagy is the dissipation of the mitochondrial transmembrane potential ($\Delta\Psi$m). Specifically, a drop in $\Delta\Psi$m is sensed as the initiating step in a series of ubiquitization events and recruitment of autophagy genes, which ultimately result in the selective degradation of damaged mitochondria (Green et al., 2011). Changes in $\Delta\Psi$m can result from the generation of pores at the outer and/or the inner mitochondrial membranes (by action of the Bcl-2 factors BAX/BAX and the mitochondrial membrane permeability transition complex MTP, respectively). Alternatively, hypoxia may also promote $\Delta\Psi$m depolarization and mitochondrial degradation at least in part by inducing BNIP3, another Bcl-1 family member (Semenza, 2009; Zhang and Ney, 2009).

Estabilization of PINK1 and recruitment of PARKIN at the outer mitochondrial membrane

The main known E3-ligase involved in mitophagy is PARKIN (parkinson protein 2, PARK2). This is a cytosolic protein that in healthy cells localizes in the cytosol (Jin and Youle, 2012). The recruitment of PARKIN to the mitochondria requires the presence of an outer membrane protein termed PINK1 (Jones, 2010; Lazarou et al., 2012). Interestingly, these PARKIN–PINK1 interactions do not occur in healthy mitochondria (Jin et al., 2010; Narendra et al., 2010). In these conditions, PINK1 is cleaved by mitochondrial proteases, including MPP or PARL (Greene et al., 2012).

PARKIN-dependent ubiquitination

PARKIN has the peculiarity of inducing K27 and K63 polyubiquitin chains, at least on mitochondrial proteins such the voltage-dependent anion channel, VDAC (Geisler et al., 2010). This differs from the canonical G76–K48 ubiquitin linkages characteristic of proteins destined for proteasomal degradation. Instead, K27 and K63 polyubiquitin chains appear to serve two purposes: first, to tether clusters of dysfunctional mitochondria together and second, to target these structures for lysosomal localization and autophagic degradation (Geisler et al., 2010).

In addition to VDAC, PARKIN ubiquitylates numerous outer mitochondrial membrane (OMM) proteins. These ubiquitylated proteins in turn act as hubs to recruit yet other factors to mitochondria to initiate mitophagy (Ashrafi and Schwarz, 2012; Chan and Chan, 2011; Chan et al., 2011). Substrates of endogenous PARKIN include mitofusin (MFN) 1 and 2, which are degraded to prevent mitochondrial fusion (Gegg et al., 2010; Poole et al., 2010). Other PARKIN ubiquitylated proteins include some components of the translocase of the outer mitochondria membrane complex (TOM70, TOM40, and TOM20), the pro-apoptotic factor BAK, and the fission protein FIS1 (Chan et al., 2011; Yoshii et al., 2011). As for non-mitochondrial proteins, HDAC6 has also been found ubiquitinated by PARKIN during mitophagy (Lee et al., 2010). Interestingly, PINK1 and PARKIN can also keep damaged mitochondria under quarantine (Kane and Youle, 2011). This occurs by means of inactivating the RhoGTPase MIRO, which is involved in the transport of mitochondria to autophagomes via kinesin complexes (Wang et al., 2011).

Selective engulfment of mitochondria

The precise mechanisms involved in the engulfment of mitochondria in autophagosomes are unclear. Initially, the p62 protein was considered to play a key role by its ability to bind both ubiquitinated proteins and the LC3 autophagosome marker. The specific contribution of p62 in mitophagy, however, is controversial (Narendra et al., 2010). Additional modulators may include AMBRA1, another target of PARKIN and an activator of class III phosphatidylinositol 3-kinase (PtdIns3K) (Van Humbeeck et al., 2011). Another mitochondrial protein involved in the recognition of LC3 is FUNCD1 (Liu et al., 2012a). Furthermore, low ATP content (sensed by AMPK and ULK1), starvation (via HMGB1), and DNA damage (through ATM) can also impinge on autophagosome formation during mitophagy (Egan et al., 2011; Kang et al., 2011; Valentin-Vega et al., 2011).

Regarding autophagy genes, in yeast, Atg32 has been reported as a key as 'mitophagy' receptor, with 20 additional genes further contributing to the mitophagy process (Wang and Klionsky, 2011). The ATG32 homologue in mammalian cells has not been identified, but reports have linked ATG3 and ATG12 to selective mitochondrial degradation. In erythrocytes where mitochondria are completely eliminated, mitophagy is specifically driven by NIX (Ding et al., 2010; Mortensen et al., 2010). None of these genes has been analyzed in melanocytes or melanoma cells.

What is on the horizon for mitophagy and melanoma?

Macroautophagy has been abundantly linked to cancer cells, where it can display a still not completely understood dual role as a pro- or anti-tumorigenic driver depending on the cellular context (Kepp et al., 2011; Kroemer et al., 2010). Melanoma is no exception, and examples of active autophagosome/lysosome programs have also being demonstrated in vitro and in vivo (reviewed in (Checinska and Soengas, 2012). Still, when this perspective went into press, a PubMed search identified just a single article including the wording mitophagy in melanoma (Zanchetta et al., 2012). This study corresponded to the analysis of global aspects of mitochondrial and lysosomal morphology and distribution in the amelanotic melanoma cell line A375 prior and after treatment with simulated solar irradiation (SSI). Using the fluorescent dyes Mitotracker and Lysotracker to stain mitochondria and acidic compartments, respectively, the authors conclude that SSI promotes mitochondrial fusion with no overt mitophagy. Mitochondrial fusion was also

favored by incubation with melanin (10 mg/l), or with high doses of glutamine (6 mM). These compounds were tested as examples of an antioxidant and key building block in the Krebs cycle, respectively. Instead, the generation of SSI-driven tubular mitochondrial networks was inhibited by (i) trifluorocarbonylcyanide phenylhydrazone (0.5 mM), an uncoupler of oxidative phosphorylation, (ii) creatin (100 mM), used to mimic a fuel anaerobic ATP production, and (iii) low glutamine (0.6 mM). No kinetic or mechanistic analyses of mitochondrial degradation were performed, and the impact of ROS, mitochondrial respiration, and ATP production in melanoma cell proliferation and viability are still pending. Still, this first report supports a dynamic behavior of mitochondria in melanoma cells that grants future investigation. Translationally relevant information emerging from other cell types can serve as a guideline. Nevertheless, unique features of melanocytic cells in the context of ROS control and metabolic regulation are likely to yet again surprise the scientific community by novel mechanisms of tumor progression and response to therapy. To follow is the rationale behind some areas of research that could be related to or benefit from in-depth analyses of the interplay between biogenesis and controlled degradation of mitochondria in benign nevi and malignant melanoma.

ROS, UV, and melanoma progression

As indicated previously, deregulated ROS is a main driver of mitophagy. Given the idiosyncratic ability of melanocytic cells to generate and counteract free radicals (Afanas'ev, 2010; Fried and Arbiser, 2008; Meyskens et al., 2001), it is tempting to speculate that this malignancy represents an ideal scenario to define the physiological contribution of targeted mitochondrial degradation to tumor initiation and maintenance. Specifically, in the context of ROS production, one of the main liabilities of melanoma cells stems from one of their key defining features, namely, melanin/melanosome biogenesis (Farmer et al., 2003; Fruehauf and Trapp, 2008; Riley, 1985; Wittgen and Kempen, 2007). As the case of mitochondrial respiration, melanin production and maturation in melanosomes also involves an inherent production of various free radicals (quinones and other phenolic substrates). In normal melanocytes, melanin acts as a scavenger of UV- and intracellularly generated ROS, preventing potentially hazardous DNA damage and protein oxidation (Kadekaro et al., 2003). Instead, these protective functions may be lost in melanoma cells, at least in part, resulting from excessive oxidation of melanin itself (Farmer et al., 2003; Meyskens et al., 2001; Riley, 2003). Moreover, the production of ROS in melanoma cells is further enhanced by oncogenic activation (Cheng et al., 2004; Ferraro et al., 2006; Leikam et al., 2008), inflammation, glycolytic respiration (Meyskens et al., 2007), and deregulation of oxidoreductases such as NQO1 (Garate et al., 2010), NAD(P)H quinone oxidoreductase (Brar et al., 2001), or the mitochondrial mnSOD (Afanas'ev, 2010), among others. In addition, a variety of melanoma-associated defects on signaling cascades depending on MAPK, AKT, NOTCH1, and NF-κB affect or are modulated by ROS production (Govindarajan et al., 2007; Pinnix and Herlyn, 2007; Ueda and Richmond, 2006; Verhaegen et al., 2006). To which extent each of these ROS-associated cascades impinge on melanoma mitophagy, and moreover whether mitochondrial clearance is quantitatively and qualitatively equivalent in melanocytes and melanoma cells,—and by extension, in nevi and melanoma specimens in vivo – deserve attention.

Hypoxia

Perhaps one of the most direct arguments in support of studying the contribution of the oxygen concentration to mitochondrial metabolism in normal and malignant melanocytic cells is the observation that the epidermal compartment of the skin is mildly hypoxic. Using mouse models and cultured cells, low oxygen has been found to favor melanocyte transformation by oncogenic Ras and Akt (Bedogni et al., 2005). Participating mechanisms involve HIF-1α and the induction of survival signals by mTOR, NOTCH1, and NF-κB (Bedogni et al., 2008). Intriguingly, while HIF-1α can favor mitophagy (e.g., via BNIP3 or FUNCD1 as summarized earlier), mTOR is a classical inhibitor of autophagy (Liu et al., 2012a). Therefore, addressing the interplay between HIF1α and mTOR in mitochondrial dynamics is likely to provide new insights to melanoma progression and to the field of intracellular organelle degradation. Putative interactions between hypoxia and DNA damaging agents are also granted, considering that genotoxic stress can induce the mobilization of factors such as ATF2 to promote the depolarization of $\Delta\Psi$m in melanoma cells (Lau and Ronai, 2012; Lau et al., 2012).

Of note, HIF-1α may be induced in melanoma cells not only under markedly reduced oxygen concentrations. Recent studies have shown that the RING finger ligase SIAH2 can promote the accumulation of HIF-1α under normoxic to mild hypoxic conditions (Qi et al., 2008). SIAH2 also modulates JNK, p38, and NF-κB signaling pathways, all playing important roles in melanoma progression (Nakayama et al., 2009). Furthermore, SIAH2 can promote mitochondrial fission in other systems (Kim et al., 2011). If this were the case also in melanoma, these results would open new avenues of research with direct translational implications.

Cellular metabolism

Metabolic rewiring, a hallmark of cancer (Hanahan and Weinberg, 2011), is not foreign to melanoma. In culture, metabolic profiling and flux balance analyses have demonstrated the classical Warburg effect, whereby melanoma cells consume more glucose and produced more lactate than normal melanocytes (Scott et al., 2011). A relatively large amount of this glycolytic carbon may be diverted into serine and glycine metabolism through

phosphoglycerate dehydrogenase (Locasale et al., 2011). However, the tricarboxylic acid (TCA) cycle was functional in all melanoma lines tested, even under hypoxia, suggesting that melanoma cells are not strictly glycolytic (Scott et al., 2011). In fact, under low oxygen, melanoma cells may run the TCA in reverse, through reductive carboxylation, (using glutamine, instead of glucose, as the source or carbon) (Filipp et al., 2012). Melanomas may consequently be particularly dependent on proper mitochondrial homeostasis to maintain their malignant potential.

The LKB1-AMPK signaling pathway may also provide novel basic and translational information to the melanoma field. This is a metabolically relevant signaling cascade that acts as critical cellular sensor coupling mitochondrial respiration and energy homeostasis to cell growth, proliferation, and survival (Jansen et al., 2009). Yet, recent reports have shown that AMPK is suppressed in $BRAF^{V600E}$-expressing melanoma cells. This inhibition results from the inability of AMPK to respond to LKB1, which in these cells is inactivated by BRAF-ERK/RSK-driven phosphorylation (Zheng et al., 2009). Additional connections between dysfunctional LKB1 and melanoma progression are emerging from mouse models (Liu et al., 2012b), broadening the putative physiological conditions where metabolic regulation and mitochondrial biogenesis/degradation may contribute to tumor development. Curiously, while AMPK function may be dampened in melanoma cells, NUAK2 (an AMPK like kinase) is overexpressed and hyperactivated, particularly in acral cases (Namiki et al., 2011). The relative contribution of AMPK inhibition and NUAK2 inactivation to the metabolic intricacies of malignant melanoma cells has still to be defined. Similar open questions remain for the interplay between BRAF/ERK and mTOR, as their impact on autophagy driven by glucose or aminoacid deprivation is dependent on the cellular context (Checinska and Soengas, 2012).

Inflammation

It addition to its archetypical function in oxidative phosphorylation, mitochondria are also a central mediator in inflammatory responses (Cloonan and Choi, 2011). Mitochondrial-released ROS can influence the transactivation of pro-inflammatory factors by NF-κB or by inflammasomes (Green et al., 2011). Inflammasomes are macromolecular complexes composed of various proteins including NOD-like receptors (NLRP), the adaptor ASC, and the caspase-1 (Rathinam et al., 2012). Active caspase-1 in turn leads to the processing of IL-1β, a key cytokine with pleiotropic roles in the activation of immune responses. However, IL-1β can modulate tumor cell proliferation, invasion and metastasis, by acting in an autocrine and paracrine manner (Apte et al., 2006; Strowig et al., 2012). Therefore, the rate of mitochondrial biosynthesis and degradation can ultimately affect various aspects of tumor progression. Whether this is the case in melanoma is unknown. Nevertheless, it is relevant to note that NF-κB and inflammasomes are hyperactivated in melanoma cells (Okamoto et al., 2010; Richmond et al., 2009). Moreover, SNP analyses have identified polymorphisms in NLRP3/NLRP1 that confer melanoma susceptibility (Verma et al., 2012).

Additional argument to support in-depth analyses of selective mitochondrial degradation in melanoma is that these organelles are at the crossroads of immunity and various stress programs (Deretic, 2012). In particular, the mitochondrial antiviral signaling protein (MAVS) appears appealing. MAVS acts downstream of pattern recognition receptors (PRR) in innate immunity responses and can coordinate apoptotic and metabolic functions by associating with peroxisomes, the endoplasmic reticulum, and autophagosomes (Belgnaoui et al., 2011). Among the various pattern recognition receptors, the Toll-like receptor 3 (TLR3) and the melanoma differentiation-associated protein 5 (MDA5) bear important relevance to melanoma. Both, TLR3 and MDA5, are expressed by melanoma cells and can promote their killing (Kang et al., 2002; Salaun et al., 2007). In the case of MDA5, therapeutic activation of melanoma cell death can be induced by nanocomplexes of dsRNA that mimic viral infection. These nanocomplexes activate interferon responses, mobilize the endosomal machinery (Alonso-Curbelo and Soengas, 2009; Tormo et al., 2009b), and in an MDA5-dependent manner, elicit macroautophagy and apoptotic programs (Tormo et al., 2009). Whether or not mitochondria get degraded in a selective manner or as result of unspecific macroautophagy, and ultimately, the contribution of mitochondrial dysfunction to MDA5 (and TLR3)-driven cell death remains to be determined.

Tumor-stroma crosstalk

The evidence for bidirectional interactions of tumor cells with their surrounding stroma are abundant, extending as well to aggressive melanomas. Recent reports describing the ability of melanoma cells to educate bone marrow progenitors (Peinado et al., 2012) or the secretion of growth factors by stromal compartments modulating melanoma resistance to BRAF inhibitors (Straussman et al., 2012) are just some examples that illustrate the functional impact of the crosstalk between cancer cells and their microenvironment. However, a concept that bears relevance to mitophagy and that has not yet been explored in melanoma is the so-called autophagic tumor-stroma model of cancer cell metabolism (Lisanti et al., 2010). According to this model, tumor-released ROS induce oxidative stress in adjacent fibroblasts and other stromal cells. This ROS production would therefore mimic a state of hypoxia even in aerobic conditions. Stromal cells may then respond at two levels: first, by producing more ROS, which can in turn promote DNA damage and aneuploidy in the malignant cells and secondly, stromal cells can enter in a 'reverse Warburg effect' whereby they can undergo autophagy/mitophagy and aerobic

glycolysis. As a result, lactate, ketones, and other high-energy nutrients are produced that can be incorporated by tumor cells to fuel mitochondrial biogenesis and oxidative respiration (Witkiewicz et al., 2011). Is this would be the case in melanoma, novel therapeutic interventions could be envisioned.

Mitochondria and anticancer treatment

Mitochondrial has been a prime target of drug development because the identification of apoptotic programs acting downstream of key tumor suppressors such as p53 (Lowe et al., 1993, 1994). The finding that intrinsic mitochondrial death pathways are deactivated during neoplastic transformation (Green and Kroemer, 2004) prompted a series of pharmacological strategies to restore apoptotic competency. Particular attention has been dedicated to BH3 mimetics (antagonists of anti-apoptotic Bcl-2 family members), and other agents aimed to dissipate the $\Delta\Psi$m (by altering the mitochondrial outer membrane permeability, or promoting mitochondrial permeability transition) [see (Barbosa et al., 2012; Fulda et al., 2010) for excellent reviews]. Other efforts include compounds to boost ROS production in tumor cells, by uncoupling the respiratory chain or by blunting antioxidant defenses (Baker et al., 2007; Batinic-Haberle et al., 2010; Emadi and Gore, 2010; Fulda and Kroemer, 2011; Huang et al., 2000; Trachootham et al., 2006). More recently, metabolic reprogramming linked to or associated with the Warburg effect has attracted attention as a window for therapeutic intervention (Dang, 2012). Inhibitors of aerobic glycolysis and agents that exploit the high demand of tumor cells for lipid and membrane components are also being actively tested (Fulda et al., 2010; Levine and Puzio-Kuter, 2010). The implementation of these mitochondrial-targeting treatments is however not straightforward. Limiting factors include secondary toxicities to normal cells and counteractive mechanisms engaged by tumor cells. In particular, macroautophagy is emerging as a key culprit of induced or acquired resistance to anticancer agents (Dewaele et al., 2010; Dikic et al., 2010). The extent to which specific removal of mitochondria by mitophagy contributes or aids in drug resistance (as opposed to bulk degradation of damaged organelles) has still to be defined. Answering this question in melanoma is of relevance, considering that an increasing list of compounds promote features of autophagy, most frequently to prevent or delay cell death (Checinska and Soengas, 2012).

While it is clear that (macro)autophagy represents a main survival factor under basal and drug-induced stress, it is also evident that excessive depletion of cellular organelles is incompatible with cell viability (Dikic et al., 2010; Janku et al., 2011; Kroemer et al., 2010; Mathew and White, 2011). Examples of cell demise following treatments with novel anticancer agents can also be found in the melanoma literature [reviewed in (Checinska and Soengas, 2012)]. Intriguingly, potent stimulators of melanoma self-degradation such as nanocomplexes of synthetic dsRNA involve also concerted actions of LC3-driven autophagy and mitochondrial pro-apoptotic factors of the Bcl-2 family (NOXA) (Alonso-Curbelo and Soengas, 2009; Tormo et al., 2009b). These results emphasize again the central role of mitochondria as a rheostat defining cell fate.

Concluding remarks

Molecular and translational analyses of mitochondrial physiology are revealing an intricate array of functions that place this organelle at the central stage of survival/death decisions. This Dr. Jekyll and Mr. Hyde duality is derived from the key role of mitochondria as a cellular powerhouse, while representing a main weapon for cellular suicide (Nunnari and Suomalainen, 2012; Scherz-Shouval and Elazar, 2010). Defective mitochondria are at the heart of aging and a variety of pathologies ranging from neurodegeneration to a plethora of metabolic diseases (Copeland, 2011; Galloway and Yoon, 2012). Moreover, famed cancer hallmarks related to unlimited proliferative capacity, evasion of growth suppressors, pro-angiogenic behavior and defects in cell death, are intimately related to mitochondrial biology (Galluzzi et al., 2009) (Hanahan and Weinberg, 2011). Similarly, inflammation and the metabolic reprogramming characteristic of cancer cells also rely on structural and mechanistic changes in the mitochondrial network (Dang, 2012). Puzzlingly, despite being so critical to other tumor types, specific contribution of mitochondrial homeostasis to melanoma progression and drug response remains largely unknown. This perspective has focused on the least unexplored aspect of mitochondria in melanoma, namely, its selective degradation by mitophagy. As a virtually virgin field, opportunities for senior and junior scientists are vast. In addition to the multiple open questions indicated previously, the landscape of UV-driven mutations in melanoma cells, and their putative crosstalk with ROS and hypoxia (main drivers of mitophagy), offer an exciting platform for basic and clinically oriented research. Given the progressive oxidation of melanin during melanoma progression, it would be interesting to define the specific wiring of mitophagy programs in senescent benign nevi, with respect to transformed melanocytes in the process of cell invasion and metastasis. To which extent the inherent heterogeneity and plasticity of melanoma cells [and their microenvironment (Bailey et al., 2012; Hoek and Goding, 2010; Roesch et al., 2010; Villanueva and Herlyn, 2008)] is related to mitochondrial half live in vivo also deserves attention. Similarly, a better understanding of mitochondrial dynamics may prove relevant in the context of clinical trials. For example, it is tempting to speculate that biphasic responses to anticancer agents (sensitivity versus resistance phases) reflect differential rates apoptosis, bulk autophagy, and controlled mitophagy. Finally, drug

developing efforts may take advance of the unique features of the mitochondrial membrane potential, $\Delta\Psi m$. Targeted delivery to the mitochondria has already being achieved for imaging purposes (D'Souza et al., 2010; Weissig, 2011). Moreover, series of peptide- or vesicle-based carriers are being developed, which may ultimately represent an alternative to exploit the dark side of mitochondrial death programs (Fulda et al., 2010; Malhi and Murthy, 2012; Weissig, 2011). Understanding how to exploit the hidden armamentarium in the mitochondria of melanoma cells may thus help move this field forward toward more efficient therapeutic responses.

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