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Review

Prohibitin Ligands in Cell Death and Survival: Mode of Action and Therapeutic Potential

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Prohibitins (PHBs) are scaffold proteins that modulate many signaling pathways controlling cell survival, metabolism, and inflammation. Several drugs that target PHBs have been identified and evaluated for various clinical applications. Preclinical and clinical studies indicate that these PHB ligands may be useful in oncology, cardiology, and neurology, as well as against obesity. This review covers the physiological role of PHBs in health and diseases and current developments concerning PHB ligands.

Introduction

Prohibitin-1 (PHB1, formerly known as BAP32) and its homolog PHB2 (formerly BAP37, REA, or prohibitone) are pleiotropic proteins with multiple functions. PHB1 was initially identified in 1989 by McClung and collaborators by screening for potential tumor suppressors with antiproliferative activities that were highly expressed in normal resting, but not regenerating, rat liver (McClung et al., 1989; Nuell et al., 1991). PHBs have since been shown to act as a hub for many signaling pathways triggered by growth factors, the immune response, and steroid hormones regulating metabolism, mitochondrial biogenesis, cell migration, division, and survival.

PHBs are composed of an N-terminal transmembrane domain, an evolutionarily conserved PHB domain that is common to other scaffold proteins (including stomatin, flotillin, and HflK/C), and a C-terminal coiled-coil domain that is involved in the interaction between PHB1 and PHB2 (Figure S1 available online). Although the structure of PHBs has not been solved, Winter et al. (2007) were able to generate structural models of human PHBs based on the known structure of flotillin-2, which belongs to the same family of membrane proteins.

Mitochondrial Functions of PHBs

Located in the mitochondrial inner membrane, PHB1 and PHB2 interact with each other to form heterodimers that are organized in ring-like structures with a diameter of 20–25 nm (Figure 1; Back et al., 2002; Coates et al., 2001). These supercomplexes maintain the structure of mitochondria and regulate their functions (Tatsuta et al., 2005). PHBs protect newly imported proteins from degradation by the m-AAA (mitochondrial ATPases Associated with diverse cellular Activities) protease (Steglich et al., 1999), promote mitochondrial protein synthesis (He et al., 2012), maintain the organization and copy number of mitochondrial DNA (mtDNA; Kasashima et al., 2008), and act as a chaperones for newly synthesized proteins of the mitochondrial complex I (Nijtmans et al., 2000, 2002) and the GTPase optic atrophy 1 (Opa1) during mitochondrial fission and morphogenesis (Merkwirth et al., 2008). PHB1 also interacts with the adaptor protein p66Shc, a major mediator of stress-induced apoptosis (Madireddi, 2006). This adaptor protein is activated by UVC, H_2O_2, and reactive oxygen species (ROS), and induces the generation of ROS in mitochondria, leading to apoptosis (Gertz et al., 2008). It remains unclear whether PHB1 modulates this proapoptotic response; nevertheless, it is tempting to speculate that p66Shc is involved in the cytoprotective activities of PHBs and their ligands, particularly against oxidative stress (Bernard et al., 2011; Fahrig et al., 2005; Kathiria et al., 2012a; Liu, et al., 2009b; Ribeiro et al., 2012a).

PHB2 is also regulated by a second messenger, sphingosine-1-phosphate (S1P), which controls diverse physiological processes through several targets. S1P binds with high affinity to PHB2 and thereby maintains the integrity of the mitochondrial respiration machinery (Strub et al., 2011). This process is involved in the preconditioning protection of the heart against ischemia-reperfusion injury (Gomez et al., 2011).

Regulation and Physiological Role of PHBs

PHBs are not limited to mitochondria; they are also found in the nucleus, the endoplasmic reticulum, the plasma membrane, and macrophage phagosomes (Garin et al., 2001), where they modulate many aspects of cell physiology. Their roles are extremely complex, partly because PHBs are themselves regulated by several tyrosine and serine phosphorylations, *O*-Glc*N*Ac modifications, palmitoylations, transamidations, and tyrosine nitrosylations (Mishra et al., 2010). PHB1 interacts physically with the second messenger PIP3, and PHB2 interacts with S1P (Ande and Mishra, 2009; Strub et al., 2011).

Each posttranslational modification of the PHBs has substantial effects on their activity (Figure 2). One of the best-documented examples is Akt phosphorylation of PHB1 at Thr258, which blocks its interaction with Shp1/2 and facilitates Akt signaling (Ande and Mishra, 2009). Shp1/2 is a phosphatase that facilitates Akt signaling and enhances insulin signaling. Additionally, the phosphorylation of PHB1 at Thr258 is necessary for the activation of C-Raf (Raf-1) by Ras (Chiu et al., 2013). Rajalingam et al. (2005) established that the activation of C-Raf by Ras requires the heterodimerization of phospho-PHB1(Thr258) with C-Raf. The phosphorylation of PHB1 at

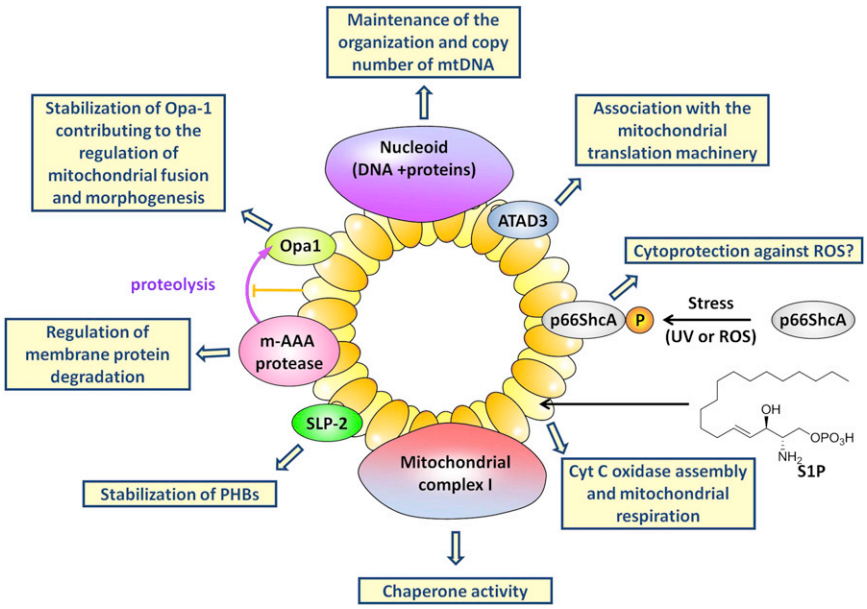

Figure 1. Function of PHB1 and PHB2 in Mitochondria

In the mitochondrial inner membrane, PHB1 and PHB2 are organized in ring-like structures that maintain the structure and regulate the functions of mitochondria through interaction with OPA-1, m-AAA protease, SLP-2 (stomatin-like protein 2), ATAD3 (ATPase family AAA Domain-containing protein 3), and mtDNA.

Thr258 in the plasma membrane of cancer cells activates PI3K/Akt and C-Raf/ERK pathways, which promote proliferation and metastasis (Chiu et al., 2012). By contrast, insulin-receptor-induced phosphorylation of PHB1 at Tyr114 promotes its heterodimerization with the phosphatase Shp1 and blocks Akt signaling (Ande et al., 2009a).

It has been suggested that the regulation of PHB1 by transforming growth factor β (TGF-β) is responsible for the dual effect of this cytokine in prostate cancer (Zhu et al., 2010). In the early stage of tumorigenesis, TGF-β acts as a tumor suppressor, but subsequently it promotes metastatic spread during cancer progression. The binding of TGF-β to its receptor triggers the C-Raf/MEK/ERK pathway and also activates Smad 2/3 or Smad 1/5, thereby causing opposite effects (Figure 2). ERK activates protein kinase C δ (PKC-δ), which leads to the phosphorylation of PHB1 and consequently to cell survival and invasion. However, Smad signaling also upregulates 14-3-3 protein, which inhibits PKC-δ, leading to a hypophosphorylation of PHB1 that promotes apoptosis.

The activity of PHB2 is regulated by serine and threonine phosphorylation, in particular at Ser91 and Tyr248 (Ross et al., 2008; Sun et al., 2011). Calcium/calmodulin-dependent protein kinase IV (CaMKIV) phosphorylates PHB2 at Ser91, impeding its ability to repress the transcriptional activity of myocyte enhancer factor 2 (MEF2; Sun et al., 2011). The consequence of this phosphorylation of PHB2 on the activity of other transcription factors has not been reported. PHB2 also inhibits circadian transcription by interacting with casein kinase 1 ε (CK1ε; Kategaya et al., 2012).

PHBs have been detected at the surface of platelets, microglial cells (Wintachai et al., 2012), endothelial cells in adipose tissue (Kolonin et al., 2004), paclitaxel-resistant cancer cells (Patel et al., 2010), intestinal epithelial cells (Sharma and Qadri, 2004), and activated T cells (Yurugi et al., 2012). In platelets, PHB1 and PHB2 interact directly with protease-activated receptor 1 (PAR1) to promote platelet aggregation (Zhang et al., 2012). The physiological role of PHB1 at the surface of microglial cells and endothelial cells of adipose tissue remains elusive, but it has been demonstrated that PHB1 at the surface of cancer cells is involved in resistance to paclitaxel (Patel et al., 2010). A virulence antigen of *Salmonella typhi*, the causative agent of typhoid, interacts with PHB1 and PHB2 at the surface of intestinal epithelial cells, resulting in downregulation of the mitogen-activated protein kinase (MAPK) cascade and inhibition of early inflammatory responses (Patel et al., 2010). Similarly, considerable proportions of total PHB1 and PHB2 are located on the surface of activated T cells, where they contribute to the activation of ERK induced by T cell activation (Yurugi et al., 2012).

PHB1 is also present in human serum, embedded in lipid droplets where it interacts with the complement protein C3 to promote innate immunity (Mishra et al., 2007). Interestingly, serum PHB1 and PHB2 concentrations were found to be significantly elevated in patients with colorectal cancer, suggesting that they may serve as a biomarker for this type of cancer (Mengwasser et al., 2004).

A brief presentation of the interactions between PHBs and other proteins is provided in Table 1 (for a list of other putative partners of PHB1, see Table S1).

PHBs and Cancer

Both PHB1 and PHB2 are involved in growth, resistance to chemotherapy, and metastasis through several mechanisms, including activation of the Ras-C-Raf-MEK-ERK pathway, modulation of TGF-β signaling, and transcriptional regulation. Indeed, PHB1 interacts with the transcription factor p53 in the nucleus of cancer cells to increase its transcriptional activity (Fusaro et al., 2003). p53 is a tumor suppressor that controls the cell cycle, apoptosis, genomic stability, and angiogenesis (Chander et al., 2010, 2011; Fusaro et al., 2003; Joshi et al., 2007). A component of ubiquitin ligase complexes, Skp2B, which is overexpressed in many breast cancers, interacts with both PHB1 and PHB2 to promote their degradation (Chander et al., 2010, 2011; Umanskaya et al., 2007). Because PHB1 acts as an activator (Fusaro et al., 2003) and chaperone (Chander et al., 2011) for p53, this depletion in PHB1 leads to an attenuated activity of p53 in cancer cells overexpressing Skp2B and consequently promotes their survival.

PHB1 is also an important regulator of the retinoblastoma tumor suppressor protein (Rb) and its family members P107 and p130 (Wang et al., 1999b). These proteins bind and inhibit

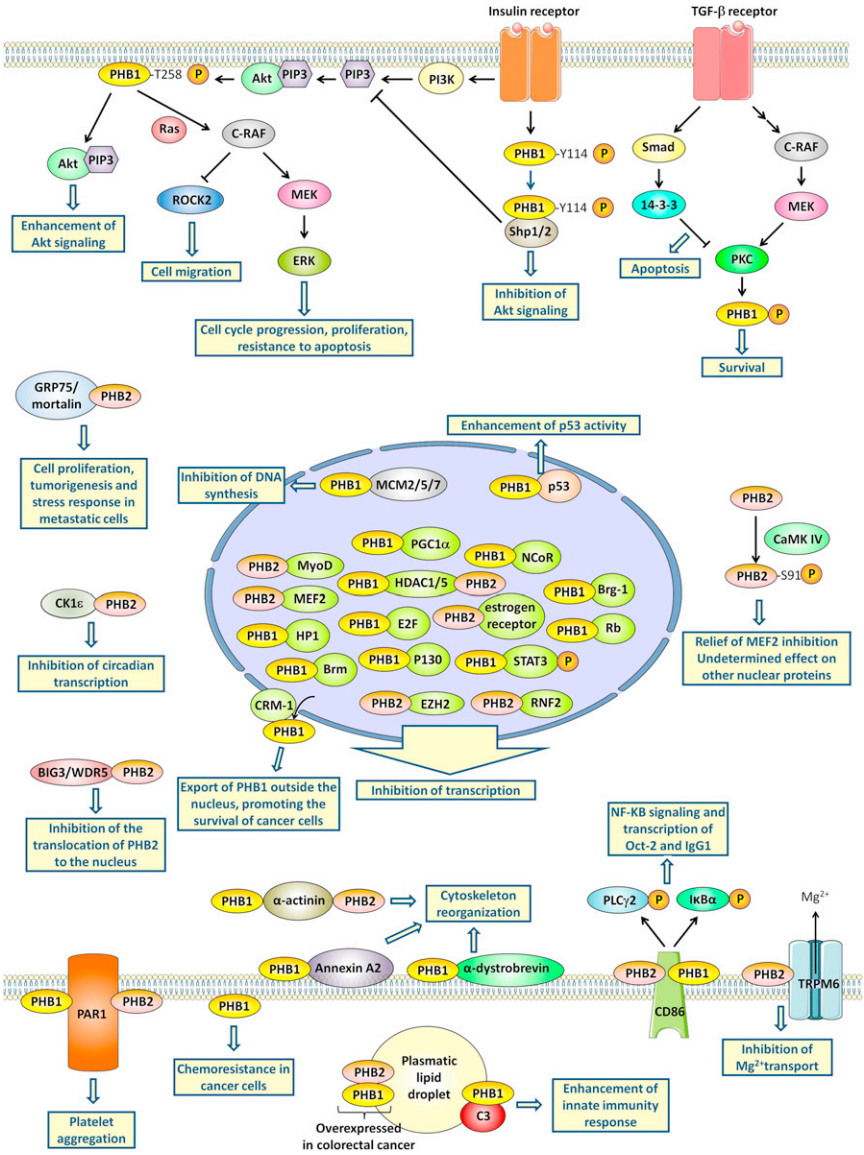

Figure 2. PHB Signaling

PHBs integrate many signaling pathways (e.g., Akt, C-RAF-MEK-ERK, CaMK, and PKC) to orchestrate various aspects of cell physiology, including metabolism, transcription, apoptosis/survival, cytoskeleton reorganization, and differentiation.

E2F transcription factors, leading to cell-cycle arrest. PHB1 represses the activity of E2F transcription factors by interacting with several proteins, including Rb, histone deacetylases (HDACs), and the nucleosome-remodeling proteins Brg-1 and Brm (Wang et al., 1999a, 1999b, 2002).

A growing body of evidence indicates that the cellular localization of PHBs is a determinant of their function, especially in cancer cells. Patel et al. (2010) found that PHB1 is overexpressed on the surface of cancer cells that are resistant to taxoids, doxorubicin, and etoposide. They also demonstrated that the redirection of PHB1 from the cytoplasm to the cell surface is critical for drug resistance, and their work suggests that cell-surface PHB1 may serve as a biomarker of chemoresistance in cancer patients.

In normal epithelial breast cells, PHB1 is located primarily in the mitochondria, but in invasive and noninvasive breast cancer cells it is mainly confined to the nucleus, suggesting that PHB1 localization is associated with tumorigenesis (Chen et al., 2011). Confocal microscopy experiments demonstrated that PHBs colocalize with the proto-oncogenes c-*myc*, c-*fos*, p53, and Rb in neuroblastoma SK-N-SH cells (Li et al., 2011).

Treatment with the anticancer drug camptothecin leads to PHB1 and p53 being exported from the nucleus to the mitochondria of breast cancer cell lines (Fusaro et al., 2003). This camptothecin-induced translocation of PHB1, which is dependent on the protein transporter CRM-1, occurs preferentially in transformed cell lines and not in untransformed or primary cells. A peptide corresponding to the nuclear export signal of PHB1 prevented the export of PHB1, resulting in the protection of cells from apoptosis, indicating that the translocation of PHB1 from the nucleus to the mitochondria contributes to its tumor-suppressive effects (Rastogi et al., 2006b). One mechanism by which the intracellular location of PHBs is controlled involves the signaling protein BIG3/WDR5, which traps PHB2 in the cytoplasm and consequently stimulates the estrogen-signaling pathway in the hormone-related growth of breast cancer cells (Kim et al., 2009).

Beyond these activities of PHBs as proteins, the 3′-UTR of PHB1 messenger RNA (mRNA) was found to suppress both the proliferation of human breast cancer cells in vitro and the growth of breast tumors in xenografted mice in vivo (Manjeshwar et al., 2003). This was the first demonstration that an RNA molecule can function as a tumor suppressor in human breast cancer.

The tumor-suppressor activity of PHB1 and its mRNA 3′-UTR is further supported by the discovery that the oncogenic microRNA-27a (miR-27a), which is upregulated in many cancers (Chhabra et al., 2010), targets the 3′-UTR of PHB1 and downregulates PHB1 in human gastric adenocarcinoma (Liu et al., 2009a). Fletcher et al. (2012) demonstrated that miR-27a is upregulated by androgen receptor in prostate cancer, resulting in reduced PHB1 mRNA and protein levels and increased cancer cell growth. Interestingly, adding PHB1 to cells overexpressing miR-27a blocked miR-27a-induced growth, demonstrating that the oncogenic activity of miR-27a strongly involves PHB1 signaling in prostate cancer.

An important question that needs to be examined is why PHBs display both anti- and protumorigenic roles in cancers (summarized in Table 2). This dichotomy may reflect the heterogeneity of cancer cell types, which are manifested by an alteration of different signaling pathways. Consequently, the regulation of transcription, translation, and posttranslational modification of PHBs probably depends on the cancerous cell type involved. These posttranslational events (e.g., phosphorylation, SUMOylation, *O*-GlcNAc modification, palmitoylation, and transamidation) modulate both the subcellular localization of PHBs and their downstream effects on proliferation and survival of cells. On the cell surface PHB1 promotes chemoresistance, but on the inner face of the plasma membrane it can contribute to the oncogenic activation of C-Raf. However, it is tempting to speculate that in the nucleus PHB1 displays antitumorigenic activity due to its action on p53, Rb, p107, p130, and components of the mammalian replication machinery. Additional studies are required to decipher the roles played by signaling and intracellular localization of PHBs in oncogenesis.

PHBs and Cytoprotection

Many studies have indicated that PHBs protect cells from oxidative stress, which is due to an excessive production or an impaired elimination of ROS (Jones, 2008). It is closely associated with mitochondrial dysfunction and is an important component of the etiology of cancers, inflammatory, cardiovascular, and neurodegenerative diseases, and diabetes mellitus. In view of the central role of PHBs in maintaining the normal structure and function of mitochondria, it is unsurprising that PHBs can alleviate the deleterious effect of oxidative stress.

Many stresses, in particular oxidative stress, upregulate PHB1 and PHB2 to promote cell survival. Indeed, PHB1 expression is enhanced in neurons by various stresses, including electrical stimulation, hypoxia-ischemia, oxygen-glucose deprivation (Zhou et al., 2012), exercise-induced neuroplasticity (Ding et al., 2006), injection of the neurotoxin 6-hydroxydopamine (Park et al., 2010), and schizophrenia-induced oligodendrocyte dysfunction (Bernstein et al., 2012). PHB1 is also upregulated in the liver of steatohepatitis patients (Tsutsumi et al., 2009), in fetal rabbit lung after exposure to hyperoxic conditions (Henschke et al., 2006), in pancreatic β-cells after ethanol intoxication, (Lee et al., 2010b), and in cardiac cells after ischemic-hypoxic preconditioning (Kim et al., 2006; Muraguchi et al., 2010) or chronic restraint stress (Liu et al., 2004). This cytoprotectant response involves the translocation of PHB1 from the nucleus and the cytoplasm to mitochondria in pancreatic β-cells (Lee et al., 2010b), in the retina and retinal epithelium (Lee et al., 2010a), and in ovarian granulosa cells (Chowdhury et al., 2007; Figure S2). Sripathi et al. (2011) suggested that the localization and trafficking of PHBs are determined by the modulation of their binding to specific lipids. Indeed, strong binding of PHB1 to cardiolipin was shown to correlate with the localization of PHB1 within mitochondria in transformed epithelial cells after an oxidative stress. By contrast, under normal conditions in these cells, PHB1 binds strongly to PIP3 but not to cardiolipin.

Overexpression of PHB1 protects pancreatic β-cells, ovarian granulosa cells, and cardiomyocytes from apoptosis induced by ethanol, ceramide, staurosporine, serum withdrawal, and oxidative stress-induced injury, consistent with PHB1 having a cytoprotective role (Chowdhury et al., 2007, 2011; Lee et al., 2010b; Liu et al., 2009b).

Merkwirth et al. (2012) recently described a critical role of PHB2 in the survival of neurons. Neuron-specific deletion of PHB2 in the mouse forebrain impaired mitochondrial architecture, leading to tau hyperphosphorylation and filament formation. This phenotype was accompanied by severe behavioral and cognitive dysfunctions that were reminiscent of Alzheimer's disease.

The details of the mechanisms of this cytoprotection remain poorly documented. The only other actors that have been identified as being involved are S1P and STAT3. S1P protects the heart from ischemia-reperfusion damage by directly activating PHB2, thereby regulating respiration and cytochrome oxidase subunit IV assembly and decreasing the heart's susceptibility to opening of the permeability transition pore (PTP; Gomez et al., 2011).

Interleukin-6 (IL-6) increases PHB1 levels through phosphorylation of the transcription factor STAT3 to promote the survival of intestinal cells and cardiomyocytes (Gratia et al., 2012; Theiss et al., 2007b). In intestinal epithelial cells in vivo, PHB1 interacts with STAT3 to modulate STAT3-mediated apoptosis (Kathiria et al., 2012b). In cardiomyocytes, IL-6-induced upregulation of PHB1 is central to the cardioprotective effects of IL-6 against oxidative stress (Gratia et al., 2012). Importantly, phosphorylated STAT3 protects cardiomyocytes against oxidative stress by stimulating respiration and inhibiting the PTP within mitochondria, and not through a transcriptional effect (Boengler et al., 2010). It would be interesting to determine whether PHB1 modulates this mitochondrial action of STAT3.

PHBs and Inflammatory Diseases

Inflammatory bowel diseases (IBDs) are characterized by strong oxidative stresses associated with downregulated antioxidant enzymes in the intestinal mucosa (Lih-Brody et al., 1996). Several studies in vitro and in vivo have indicated that PHB1 alleviates intestinal inflammation and promotes the survival of cells exposed to oxidative stress. In intestinal epithelial cells, PHB1 binds to a *Salmonella typhi* antigen to inhibit the inflammatory response to *S. typhi* infection (Sharma and Qadri, 2004). PHB1 expression is abnormally low during ulcerative colitis and Crohn's disease, the two most common forms of IBD (Hsieh et al., 2006; Theiss et al., 2007a; Yeo et al., 2006), due to an overstimulation of tumor necrosis factor α (TNF-α) signaling in the inflamed colon (Theiss et al., 2009a). This downregulation of PHB1 increases ROS signaling and TNF-α-induced autophagy, promoting inflammation in patients with IBD (Kathiria et al., 2012a). Interestingly, transgenic mice overexpressing PHB1 in intestinal epithelial cells exhibit reduced proinflammatory nuclear factor κB (NF-κB) signaling in the colonic mucosa after treatment with TNF-α, demonstrating that PHB1 plays a critical role in inflammation. A detailed examination of this mechanism of action revealed that PHB1 inhibits TNF-α-induced translocation of NF-κB by downregulating the expression of importin α3, a protein involved in NF-κB nuclear import (Theiss et al., 2009a). In an in vivo model of IBD, PHB1 transgenic mice exhibited lower oxidative stress and colitis than wild-type mice (Theiss et al., 2009b). This cytoprotective effect is due to the upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcriptional activator of

Table 1. Proteins that Interact with PHBs

Binding Partners	PHB	Biological Consequences	References
Mitochondrial Proteins			
m-AAA protease	PHB1	regulation of membrane protein degradation	Steglich et al., 1999
ATAD3	PHB1	mitochondrial protein synthesis	He et al., 2012
nucleoids (mitochondrial DNA associated to nucleoproteins)	PHB1/2	maintenance of the organization and copy number of the mtDNA	Kasashima et al., 2008
dynamin-like GTPase OPA-1	PHB1	stabilization of the structure of mitochondria during their fusion and morphogenesis	Merkwirth et al., 2008
subunits of cytochrome *c* oxidase	PHB1	function of the mitochondrial respiratory chain	Tsutsumi et al., 2009
NADH-ubiquinone oxidoreductase 30 kDa subunit	PHB1	stabilization of mitochondrial complex I	Park et al., 2010
ND4 and ND5	PHB1	assembly of mitochondrial complex I	Bourges et al., 2004
SLP-2	PHB1/2	stabilization of PHBs	Da et al., 2008
TFAM	PHB1	regulation of copy number of mitochondrial DNA	Kasashima et al., 2008
ANT2	PHB2	unknown	Kasashima et al., 2006
VDAC2	PHB2	unknown	Kasashima et al., 2006
Hax-1	PHB2	stabilization of antiapoptotic Hax-1	Kasashima et al., 2006
nitric oxide-associated protein 1 (mNOA1)	PHB1	regulation of mitochondrial protein translation and respiration	Heidler et al., 2011
phosphorylated p66ShcA	PHB1	putative protection against oxidative stress	Madireddi, 2006
Transcription Factors			
estrogen receptor	PHB1/2	repression of estrogen-receptor activity	Delage-Mourroux et al., 2000; He et al., 2008; Montano et al., 1999
E2Fs	PHB1	repression of E2F activities	Gamble et al., 2007; Joshi et al., 2003, 2007; Schneider et al., 2010
p53	PHB1	enhancement of p53-mediated transcriptional activity and chaperone activity of PHB1 for p53	Chander et al., 2011; Fusaro et al., 2003; Joshi et al., 2007
MyoD and MEF2	PHB2	repression of MyoD and MEF2 activities	Sun et al., 2004, 2011
chicken ovalbumin upstream binding transcription factors I and II	PHB2	transcriptional repression	Kurtev et al., 2004
phosphorylated STAT3	PHB1	modulation of p53- and STAT3-mediated apoptosis	Kathiria et al., 2012b
Other Nuclear Proteins			
HDAC1 and HDAC5	PHB1/2	transcriptional repression	Kurtev et al., 2004
Rb and its family members p107 and p130	PHB1	repression of E2F-mediated transcription and inhibition of cell proliferation	Wang et al., 1999a, 1999b, 2002
Brg-1 and Brm	PHB1	repression of E2F-mediated transcription and inhibition of cell proliferation	Wang et al., 2002
heterochromatin protein 1 (HP1) family proteins	PHB1	repression of E2F-mediated transcription and induction of cellular senescence	Rastogi et al., 2006a
RING finger protein 2 (RNF2)	PHB1/2	repression of the transcriptional activity of E2F1 and CP2c	Choi et al., 2008; Lee et al., 2008
histone methyltransferase EZH2	PHB2	repression of estrogen-dependent transcription	Hwang et al., 2008
PGC1α (PPARγ coactivator 1)	PHB2	inhibition of the transcriptional activity of PPARγ	Endo, 2011

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Table 1. *Continued*

Binding Partners	PHB	Biological Consequences	References
minichromosomes maintenance complex of proteins MCM2, MCM5, and MCM 7	PHB1	inhibition of DNA replication	Rizwani et al., 2009
nuclear receptor corepressor 1 (NCoR)	PHB1	transcriptional repression of E2F family members	Wang et al., 2002
Other Signaling Proteins			
IgM receptor	PBH1	phosphorylation of PHB1	Terashima et al., 1994
protease-activated receptor 1	PHB1/2	platelet aggregation	Zhang et al., 2012
C-Raf	PBH1	phosphorylation of C-Raf by Ras and consequently activation of the MEK-eIF4 pathway	Chiu et al., 2012; Chiu et al., 2013; Rajalingam et al., 2005
Akt2	PHB2	cell-cycle exit and myogenic differentiation	Héron-Milhavet et al., 2008; Sun et al., 2004
WD repeat domain 5 (WDR5, BIG3)	PHB2	inhibition of the translocation of PHB2 to the nucleus	Kim et al., 2009
Skp2B	PHB1/2	attenuation of the transcriptional activity of p53, release of the repression of the estrogen receptor	Chander et al., 2010, 2011; Umanskaya et al., 2007
MLK2 (mixed lineage kinase 2)	PHB1	unknown	Rasmussen et al., 1998
Shp1 (phosphatase)	phospho-Y^{114}-PHB1	modulation of insulin signaling	Ande et al., 2009a
PKC-δ	PHB1	phosphorylation of PHB1 leading to a decrease in the inner mitochondrial membrane permeability and to cell survival	Zhu et al., 2010
14-3-3 protein	PHB1	inhibition of PHB1 phosphorylation leading to an increase in the inner mitochondrial membrane permeability and to apoptosis	Zhu et al., 2010
α-dystrobrevin	PHB1	NB4 cell granulocytic differentiation	Borutinskaite et al., 2011
transient Receptor Potential Melastatin 6 (TRPM6)	PHB2	inhibition of TRPM6-mediated transepithelial Mg^{2+} transport	Cao et al., 2009
HSP70	PHB1	resistance to stress	Liu et al., 2010
glucose-regulated protein 75 (GRP75)/mortalin	PHB1	cell proliferation, tumorigenesis and stress response in metastatic cells	Martín et al., 2008
phospho- Ser^{291}- Syk	PHB1	B cell antigen-receptor signaling	Paris et al., 2010
CRM-1	PHB1	export of PHB1 outside of the nucleus selectively in cancer cells	Rastogi et al., 2006b
Pex14p	PHB1	regulation of the peroxisomal importomer	Oeljeklaus et al., 2012
annexin A2	PHB1/2	interaction blocked by calcium, association with lipid rafts	Zhu et al., 2010
alpha-actinin	PHB1/2	interaction blocked by calcium, association with lipid rafts	Bacher et al., 2002
EHD2	Palmitoylated PHB1	inhibition of pyruvate carboxylase leading to a decrease in glucose and fatty acid oxidation	Ande and Mishra, 2010; Vessal et al., 2006
C3	PHB1	enhancement of innate immunity response	Mishra et al., 2007
CK1ε	PHB2	inhibition of circadian transcription	Kategaya et al., 2012
Proteins of Pathogens			
SV40Tag (viral oncoprotein)	PHB1	interruption of the association between PHB1 and Brg-1/Brm	Wang et al., 2002
HIV-1 glycoprotein	PHB1/2	viral spread in nonpermissive cells	Emerson et al., 2010
DENV-2 E protein	PHB2	internalization of the dengue virus	Kuadkitkan et al., 2010

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Table 1. *Continued*

Binding Partners	PHB	Biological Consequences	References
Chikungunya virus E2 protein	PHB1	internalization of the chikungunya virus	Wintachai et al., 2012
SARS-CoV nonstructural protein nsp2	PHB1/2	supposed disruption of intracellular host signaling during SARS-CoV infection	Cornillez-Ty et al., 2009
Vi polysaccharide of *Salmonella typhi*	PHB1/2	inhibition of the inflammatory response upon infection with Vi- S. typhi.	Sharma and Qadri, 2004
capsid protein VP1 of foot-and-mouth disease virus	PHB1	dephosphorylation of PHB T258	Chiu et al., 2012
macrophage surface HSP70	*Leishmania donovani* PHB1	induction of a strong humoral response in leishmaniasis patients	Jain et al., 2010

See also Table S1.

antioxidant responses. Further evidence that PHB1 is a putative therapeutic target for treating IBD comes from the observations that adenovirus-directed administration by enema and nanoparticle-based colonic delivery of PHB1 effectively enhanced the levels of PHB1 at the surface of colonic epithelial cells and alleviated colitis induced by dextran sodium sulfate (Theiss et al., 2011).

Picard et al. (2013) recently demonstrated that PHB1 accumulates in the nucleus of osteoarthritic chondrocytes, where it represses the expression of the transcription factor PITX1, suggesting an involvement of PHB1 in the etiology of osteoarthritis.

In addition to PHB1, PHB2 is also involved in inflammation. Indeed, heterozygous PHB2$^{+/-}$ were shown to be more sensitive to liver insults and inflammatory aggressions than wild-type animals, confirming a cytoprotective and anti-inflammatory role of PHB2 in liver (Sánchez-Quiles et al., 2012).

Lucas et al. (2013) recently discovered how PHB1 and PHB2 induce an immune response in B cells. The interaction of CD86 with PHBs was shown to induce the phosphorylation of IκBα, phospholipase Cγ2, and protein kinase Cα/β(II), leading to the nuclear translocation of NF-κB (p65) into the nucleus and the subsequent transcription of Oct-2 and IgG1.

PHBs and Metabolic Diseases

Ande and Mishra (2009) and Ande et al. (2009a) established that PHB1 is a key regulator of insulin signaling and adipocyte differentiation. Indeed, PHB1 is directly phosphorylated by the insulin receptor to promote insulin signaling and block Akt signaling, whereas phosphorylation of PHB1 by Akt has the opposite effect.

Interestingly, PHB1 is not only phosphorylated but is also conjugated to *O*-linked β-*N*-acetylglucosamine in myoblast cells in response to insulin and high glucose. This glucose-induced *O*-GlcNAc modification and phosphorylation of PHB1 may be associated with insulin resistance modification and tyrosine phosphorylation of PHB (Ande et al., 2009b; Gu et al., 2011).

PHB1 inhibits pyruvate carboxylase and decreases insulin-stimulated oxidation of glucose and fatty acid in adipocytes, implying that it has a role in promoting lipid accumulation (Vessal et al., 2006). Crosslinking experiments showed that PHB1 associates with pyruvate carboxylase and Eps 15 homology domain protein 2 (EHD2), suggesting that PHB1 shuttles between the extracellular space and the mitochondria by a mechanism involving lipid rafts and EHD2.

Recently, Ande et al. (2012) demonstrated that treatment of preadipocytes with insulin or a peroxisome proliferator-activated receptor γ (PPARγ) agonist upregulates PHB1, thereby promoting preadipocyte differentiation into adipocytes. Remarkably, overexpression of PHB1 was sufficient to induce adipogenesis. PHB1 on the cell surface is also a marker of adipose vasculature and may be a possible therapeutic target for obesity (see below).

PHBs and Pathogenic Agents

Several recent studies demonstrated that the internalization of some viruses involves PHBs. For instance, PHB1 interacts with chikungunya virus (Wintachai et al., 2012), PHB2 interacts with dengue virus (Kuadkitkan et al., 2010), and both PHB1 and PHB2 interact with severe acute respiratory syndrome coronavirus (SARS-CoV; Cornillez-Ty et al., 2009). PHB1 and PHB2 also interact with the HIV-1 glycoprotein to promote replicative spread in nonpermissive cells (Emerson et al., 2010). Interestingly, recombinant capsid protein VP1 (rVP1) of foot-and-mouth disease virus dephosphorylates Akt and phospho-PHB Ttr258 in lipid rafts to downregulate C-Raf/ERK signaling and inhibit metastasis of cancer cells, both in vitro and in vivo (Chiu et al., 2012). Moreover, using high-throughput mass spectrometry, Jang et al. (2012) discovered that lipoteichoic acid, a major virulence factor of Gram-positive bacteria, binds to PHB2, implicating PHB2 in host immune responses to infections.

Drug Development Outlook

The activities of PHBs are affected by some natural products (e.g., flavaglines and aurilide), fully synthetic small molecules (e.g., melanogenin), and adipotide, a chimeric peptide that is currently in phase I clinical trial. These compounds (except for adipotide) were examined for a variety of activities before it became clear that PHBs were their molecular targets. The best-studied PHB ligands are flavaglines.

Flavaglines

Flavaglines have a unique cyclopenta[*b*]benzofuran skeleton (Ebada et al., 2011; Ribeiro et al., 2012b). Rocaglamide, the first of these compounds to be described, was isolated more than 30 years ago by King et al. (1982) from medicinal plants of the genus *aglaia* (*Meliaceae*) in Southeast Asia. Since then, ~100 other flavaglines, including rocaglaol and silvestrol, have been identified (Figure 3A; Figure S3).

Table 2. Roles of PHB Signaling in Cancer

Antitumorigenic Roles of PHBs	References
PHB1 activates the tumor suppressor p53	Chander et al., 2011; Fusaro et al., 2003; Joshi et al., 2007
PHB1 interacts with the tumors suppressors Rb, p107, and p130 to repress E2F-mediated transcription and inhibit cell proliferation	Wang et al., 1999a, 1999b, 2002
PHB1 represses DNA replication by interacting with components of the mammalian replication machinery	Rizwani et al., 2009
Protumorigenic Roles of PHBs	
PHB1 and PHB2 are necessary for the activation of C-RAF by Ras	Rajalingam et al., 2005
phosphorylated PHB1 promotes the survival of prostate cancer cells	Zhu et al., 2010
the oncomir miR-27a downregulates PHB1	Liu et al., 2010; Fletcher et al., 2012
localized on the cell surface, PHB1 mediates resistance to taxoids	Patel et al., 2010

Although flavaglines display a myriad of pharmacological effects due to their anticancer, anti-inflammatory, neuroprotective, and cardioprotective properties, it is the anticancer properties that have attracted the most attention from scientists (Ebada et al., 2011; Ribeiro et al., 2012b). At concentrations in the low-nanomolar range, flavaglines inhibit the proliferation of tumor cells, are not toxic to normal cells (Hausott et al., 2004; Ribeiro et al., 2012a; Su et al., 2006; Thuaud et al., 2009, 2011; Zhu et al., 2007), and show no sign of toxicity in mice (Bernard et al., 2011; Cencic et al., 2009; Lee et al., 1998; Thuaud et al., 2009; Zhu et al., 2009). King et al. (1982) were the first to demonstrate the antileukemic activity of rocaglamide in a mouse model. Subsequently, Ohse et al. (1996) established that flavaglines strongly inhibit protein synthesis, and Lee et al. (1998) demonstrated that these compounds delay the growth of human breast cancer cells in athymic mice. However, because the tumors were not eradicated, these investigations were interrupted. Since then, with the development of targeted therapies, approaches to the development of cytostatic compounds have changed radically (Gutierrez et al., 2009). As a consequence, there is now a renewed interest in flavaglines as potential anticancer agents.

Although flavaglines have shown significant anticancer effects in mouse models of cancer (Alinari et al., 2012; Cencic et al., 2009; Hwang et al., 2004; King et al., 1982; Lee et al., 1998; Lucas et al., 2009; Meurer-Grimes et al., 2002; Thuaud et al., 2011), their most promising potential is associated with their ability to enhance the in vivo efficacy of other anticancer drugs, and in particular to relieve the resistance of tumors to chemotherapies. Indeed, flavaglines were shown to enhance doxorubicin chemosensitivity in several mouse lymphoma models (Bordeleau et al., 2008; Cencic et al., 2009; Zhu et al., 2009). Importantly, flavaglines did not display any overt sign of toxicity in mice in these studies, consistent with previous observations.

Very recently, using affinity chromatography-coupled mass spectrometry, Polier et al. (2012) identified PHB1 and PHB2 as the direct targets of flavaglines. The binding of flavaglines to PHBs inhibits Ras-C-Raf-MEK-ERK signaling, and such signaling is essential for the survival of cancer cells (Figure 3B). C-Raf needs to interact with PHBs to be phosphorylated by Ras and thus participate in signaling (Rajalingam et al., 2005). The binding of flavaglines to PHBs prevents this interaction both in vitro and in vivo. Knockdown of either PHB1 or PHB2 with small interfering RNA (siRNA) mimicked the effects of flavaglines through inhibition of cap-dependent translation and an arrest of cell-cycle progression via a depletion of cyclin D3, CDK4, CDK6, and cdc25A. Moreover, rocaglamide strongly depleted PHB1 and phosphorylated C-Raf at the plasma membrane, suggesting that the relocation of PHBs to the membrane is involved in the mode of action of flavaglines. These various observations support the idea that flavaglines exert their anticancer effects through their action on PHBs.

These findings are of particular interest because we now have evidence that Ras-C-Raf-ERK constitutes an overactivated pathway in many human cancers, and that this pathway is a promising target for treatments in oncology (Matallanas et al., 2011; Maurer et al., 2011). Bleumink et al. (2011) showed that this inhibition of C-Raf-ERK signaling blocks cap-dependent protein synthesis. Indeed, ERK activates Mnk1, which phosphorylates eIF4E, allowing cap-dependent synthesis to occur. Few proteins require phosphorylated eIF4E for their synthesis, and most of them are involved in oncogenesis, angiogenesis, and chemoresistance. Thus, the in vivo anticancer effects of flavaglines seems to be a consequence of the inhibition of the synthesis of cyclins D1 and D3, CDK4, CDK6, cdc25A c-Myc, Bcl-2, survivin, Mcl-1, the PIM1/2 kinases, vascular endothelial growth factor (VEGF), and matrix metallopeptidase 9 (MMP9) (Bordeleau et al., 2008; Cencic et al., 2009; Nasr et al., 2013; Polier et al., 2012; Schatz et al., 2011).

Although inhibition of the Ras-C-Raf-ERK signaling pathway may be involved in the flavagline-induced suppression of cap-dependent synthesis in cells (Bleumink et al., 2011), it is more difficult to explain the observed inhibition of protein synthesis in vitro, where C-Raf, MEK, and ERK are absent. This observation suggests either that another molecular target is involved in the inhibition of translation by flavaglines or that PHBs interact with the translational machinery to control its activity.

Flavaglines induce apoptosis in cancer cells through a myriad of mechanisms (Ribeiro et al., 2012b) involving cytochrome *c* (Mi et al., 2006b; Zhu et al., 2007), apoptosis-inducing factor (Thuaud et al., 2009), caspase 12 (Thuaud et al., 2009), and the MAPKs JNK and p38 (Zhu et al., 2007, 2009). Whether these mechanisms involve PHB1 and PHB2 or another target remains to be determined. The effect of flavaglines on PHBs-interacting proteins other than C-Raf is also an issue that needs to be examined.

Silvestrol is the flavagline that has been the most studied in vivo for its anticancer properties (Alinari et al., 2012; Bordeleau et al., 2008; Cencic et al., 2009; Hwang et al., 2004; Kim et al., 2007; Mi et al., 2006a; Robert et al., 2009; Schatz et al., 2011). However, the development of this compound is severely limited by its sensitivity to P-glycoprotein-mediated multidrug resistance and its suboptimal absorption, distribution, metabolism, and excretion (ADME) characteristics (Gupta et al., 2011; Liu et al., 2012). Fortunately, structure-activity relationship (SAR)

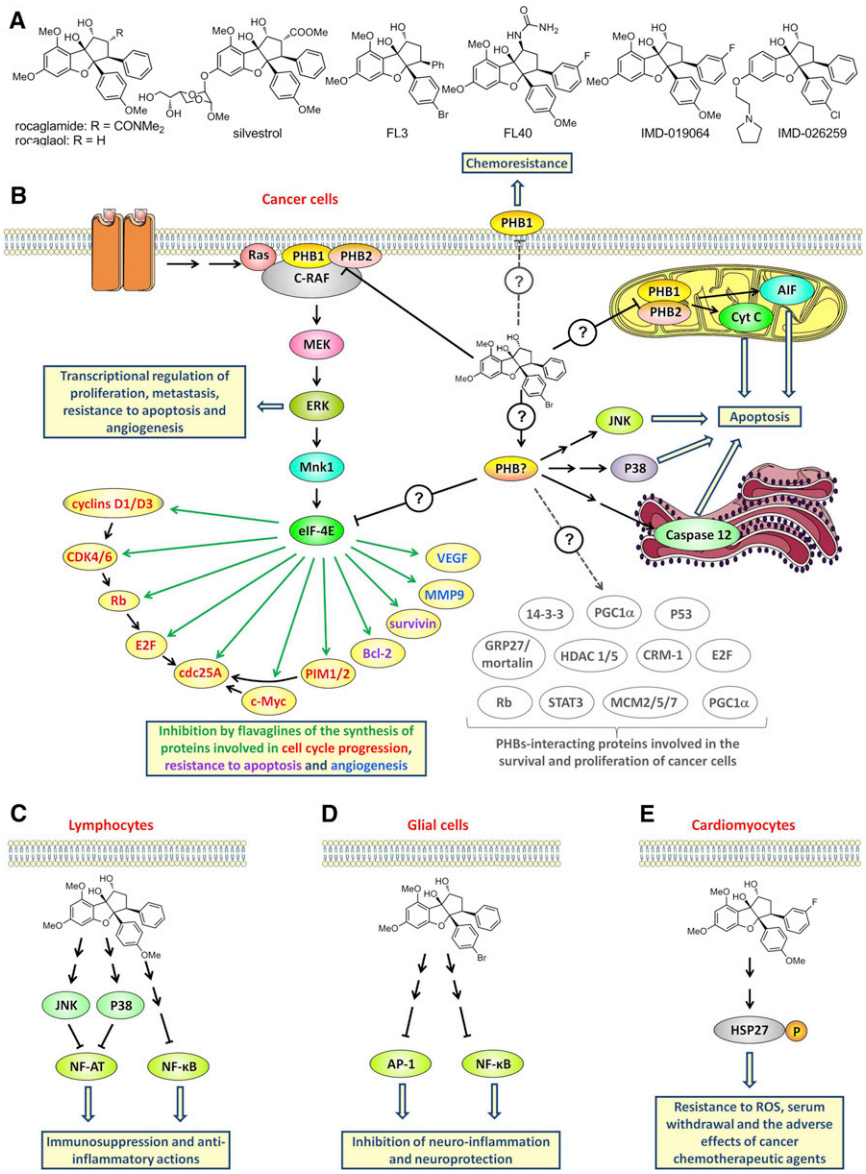

Figure 3. Structure and Pharmacological Actions of Flavaglines

(A) Structure of selected natural (rocaglamide, rocaglaol, and silvestrol) and synthetic (FL3, FL40, and IMD-019064) flavaglines.

(B–E) Proposed models of the mode of action of flavaglines in cancer cells (B), lymphocytes (C), glial cells (D), and cardiomyocytes (E).

See also Figure S2, which illustrates the translocalization of PHB1 to mitochondria in noncancerous cells to promote survival.

studies have established that the deletion of the methyl ester or the amide in position 2 results in compounds that escape this multidrug resistance without any loss of cytotoxicity (Figure S3; Ribeiro et al., 2012a; Thuaud et al., 2009, 2011).

In addition to their anticancer effects, flavaglines also display potent anti-inflammatory and neuroprotective activities. Indeed, flavaglines at nanomolar concentrations act as immunosuppressors by inhibiting the production of interferon-γ, TNF-α, IL-2, and IL-4 by T lymphocytes (Proksch et al., 2005). This immunosuppression is mediated by activation of the MAPKs JNK and p38, which selectively inactivate nuclear factor of activated T cells (NFAT; Figure 3C). It is still not known how flavaglines activate JNK and p38. At higher concentrations, flavaglines also inhibit NF-κB, another transcription factor that is involved in inflammation (Baumann et al., 2002). Interestingly, the synthetic flavagline IMD-019064 inhibits NF-κB signaling and displays potent in vitro anti-inflammatory effects that manifest as an inhibition of proinflammatory mediator release from astrocytes, microglia, and endothelial cells (Fahrig et al., 2005; Figure 3D). More importantly, IMD-019064 protects dopaminergic neurons both in vitro and in vivo in models of Parkinson's disease (MPP^+-induced neurotoxicity) and traumatic brain injury. Recent SAR studies led to the identification of FL40, which provides greater neuroprotection in vitro than IMD-019064 (Ribeiro et al., 2012a). The neuroprotective activity of flavaglines is currently being actively explored by the pharmaceutical company Intermed Discovery, which has been developing IMD-026259 (Figure 3A) for the treatment of Parkinson's disease.

These studies have prompted evaluations of the cytoprotective potential of flavaglines in other tissues. The synthetic flavagline FL3 (Figure 3A), which displays a potent cytotoxicity in cancer cells (Thuaud et al., 2009), was shown to protect cardiomyocytes in vitro and in vivo against various stresses, including anthracycline cardiotoxicity (Bernard et al., 2011). The heart is extremely sensitive to the toxicity of anthracyclines, such as doxorubicin. These medicines are among the most effective anticancer drugs available and have antitumor activity against both hematopoietic and solid tumors; unfortunately, their clinical utility is markedly hampered by their cardiotoxicity, which can lead to dilated cardiomyopathy and congestive heart failure (Minotti et al., 2004).

FL3 protects cardiomyocytes from the apoptosis induced by both doxorubicin and serum starvation (Bernard et al., 2011). Interestingly, these stresses are of different natures: doxorubicin causes an oxidative stress, whereas serum starvation blocks the growth factor signaling that is necessary for cell survival, mimicking an important component of myocardial ischemia. Treatment with FL3 significantly reduces mortality and attenuates both apoptosis and fibrosis in the hearts of doxorubicin-treated mice. Thus, flavaglines may both enhance the anticancer efficacy of anthracyclines and alleviate their main adverse effect, cardiotoxicity. Anthracyclines are central to cancer chemotherapies, so the discovery of a new class of cardioprotective agents

would be extremely valuable clinically, and further studies to examine this therapeutic application are therefore warranted.

The cardioprotective effects of flavaglines are mediated through the phosphorylation of the small heat shock protein Hsp27 (Figure 3E). Hsp27 is critical for protecting cells against many types of damage, including the cardiotoxicity of doxorubicin, and exerts its effects in several ways, including chaperone activity, control of redox homeostasis, and inhibition of apoptosis (Kostenko and Moens, 2009). Whether PHBs or another target is involved in this cardioprotective effect of flavaglines has not been examined.

Although the cytoprotective and anti-inflammatory effects of flavaglines have not yet been demonstrated to be mediated through a binding to PHBs, it is unlikely that another target is involved. Indeed, both their pharmacological effects and cytotoxicity in cancer cells occur at the same range of concentrations. As far as we know, no other natural product that possesses such a complex three-dimensional structure has been shown to bind to different classes of proteins with a comparably high affinity.

It would be useful to further evaluate this therapeutic application of flavaglines and elucidate the mechanisms involved. It may seem odd than an anticancer drug can also display various cardio- or neuroprotective effects; however, there are precedents. For example, rapamycin derivatives (Erlich et al., 2007; Khan et al., 2006; Malagelada et al., 2010) and HDAC inhibitors are both cardio- and neuroprotectants in addition to having anticancer effects (Bush and McKinsey, 2009; Mai et al., 2009). A first clue to understanding this paradox may be that PHB1 is localized mainly in the mitochondria of normal cells and in the nucleus in cancer cells (Chen et al., 2011). The translocation of PHB1 from the nucleus and cytosol to mitochondria promotes survival in normal cells but apoptosis in cancer cells (Fusaro et al., 2003). It is therefore tempting to suggest that the opposite behaviors of flavaglines in cancer and normal cells are the consequences of different intracellular localizations of PHBs.

Although many academic studies have demonstrated a therapeutic potential of flavaglines in preclinical models of cancers and inflammatory and cardiac diseases, these compounds have not yet been examined in a clinical trial. To our knowledge, only two pharmaceutical companies (Intermed Discovery and Infinity Pharmaceutical) are currently developing flavaglines for the treatment of Parkinson's disease and cancers, respectively.

Aurilide

Isolated from the Japanese sea hare (*Dolabella auricularia*; Suenaga et al., 2004; Suenaga et al., 1996), aurilide is a cyclic depsipeptide that displays a strong cytotoxicity at nanomolar and subnanomolar concentrations on a panel of cancer cell lines (Figure 4A; Figure S4). Work involving affinity chromatography identified PHB1 as the molecular target of aurilide (Sato et al., 2011). The binding of this toxin activates the proteolytic processing of the dynamin-like GTPase optic atrophy 1 (OPA1), which in turn triggers remodeling of the mitochondrial cristae, leading to apoptosis. This devastating effect on mitochondria probably explains the extreme toxicity of this compound, which impedes its study in animal models and precludes its further development as a medicament.

Melanogenin

In a quest to identify compounds that modulate skin pigmentation, Snyder et al. (2005) screened a tagged-triazine library on unpigmented melanocytes (Figure 4B; Figure S5). They identified melanogenin as an inducer of pigmentation with an EC_{50} of 2.5 μM. This compound upregulates tyrosinase, the rate-limiting enzyme in the biosynthesis of melanin.

Snyder et al. (2005) conjugated melanogenin to an agarose support, which enabled them to identify PHB1 as the molecular target by affinity chromatography. Further biological investigations confirmed that PHB1 is responsible for the induction of pigmentation. Immunofluorescence microscopy studies showed that PHB1 was localized only in mitochondria, which led these authors to suggest that the binding of melanogenin to PHB1 may disrupt the interaction between PHB1 and a transcriptional factor, thereby causing its translocation to the nucleus and induction of the expression of tyrosinase, the rate-limiting enzyme in melanogenesis.

This study was the first to demonstrate and unravel the involvement of PHB1 in the regulation of mammalian pigmentation. Although these discoveries may provide a basis for the development of novel cosmetics and drugs for the treatment of pigment disorders, no further investigation in this direction has been reported.

Recently, Wu and Wu (2012) demonstrated that PHB1 is enriched in lipid rafts in HaCaT keratinocytes after UVB irradiation, and that this protects the cells against UVB-induced apoptosis. These findings are consistent with the notion that PHB1 contributes to protection of the skin against UVB.

Adipotide

Kolonin et al. (2004) and Staquicini et al. (2011) used phage display techniques to identify a peptide homologous to a region of annexin A2 that targets PHB1 at the surface of the vascular endothelial cells of white adipose tissue (Figure 4C). By fusion with a proapoptotic sequence, they obtained adipotide (CKGGRAKDC-GG-$_D$(KLAKLAK)$_2$), a chimeric peptide that damages the vasculature of the adipose tissue and consequently disrupts the blood supply to adipocytes. In obese mice, adipotide induced a substantial loss of weight and normalized metabolism, leading to a reversal of obesity without any adverse sign of toxicity. Surprisingly, food intake was reduced, suggesting that there is an unexpected relationship between the adipose tissue vasculature and the regulation of feeding behavior (Kim et al., 2010).

In obese rhesus monkeys, 4 weeks of adipotide treatment led to a 28% reduction in body fat and also reduced food intake and improved the animals' metabolic status (i.e., reduced insulin resistance); these findings have implications for the development of treatment for type 2 diabetes (Barnhart et al., 2011). Adipotide is currently being developed by the biotech company Arrowhead Research Corporation, and entered a phase I trial involving obese patients with prostate cancer in July 2012. Because adipotide has a negative endocrine effect on tumor growth, it is expected to slow tumor growth in these patients.

Capsaicin

Capsaicin is a component of hot chili peppers that inhibits the proliferation of many cancer cell lines. Using affinity chromatography, Fletcher et al. (2012) demonstrated that capsaicin (0.1–1 mM) binds to PHB2, and that this binding induces

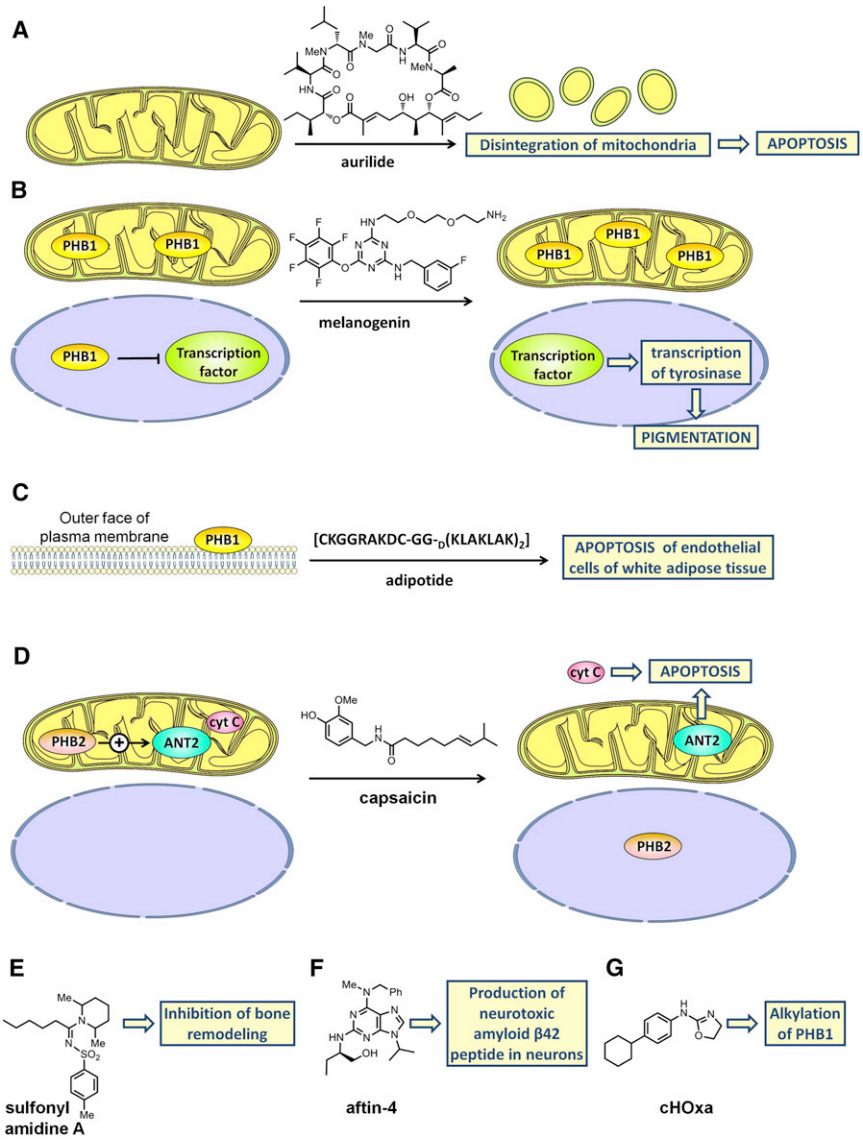

Figure 4. Proposed Mechanism of Action of Various PHB Ligands

(A) Aurilide is a cytotoxic depsipeptide that binds to PHB1 and triggers remodeling of the mitochondrial cristae to lead to apoptosis.

(B) Melanogenin binds to PHB1 to induce the expression of tyrosinase, the rate-limiting enzyme in melanogenesis.

(C) Adipotide is a chimeric peptide that targets PHB1 at the surface of the vascular endothelial cells of white adipose tissue. Its proapoptotic sequence is responsible for the damage to this tissue.

(D) Binding of capsaicin to PHB2 induces its translocation from mitochondria to the nucleus, a noncompetitive inhibition of the ANT2 and the release of cytochrome *c* into the cytosol, leading to the apoptosis of myeloid leukemia cells.

(E) Sulfonyl amidine (A) inhibits the osteoclastogenesis involved in bone remodeling. This compound binds to PHB1 and three other proteins. Which of these proteins is the relevant target involved in this antiresorptive activity remains unknown.

(F) Aftin-4, which binds to PHB1, VDAC1, and mitofilin, promotes in neurons the production of the neurotoxic peptide Aβ42, which is involved in the etiology of Alzheimer's disease.

(G) The dihydrooxazole cHOxa alkylates PHB1, suggesting that it could be used as a chemical probe to analyze the structure of PHB1.

a series of proapoptotic events in human myeloid leukemia cells, including (1) the translocation of PHB2 from mitochondria to the nucleus; (2) the dissociation of PHB2 from Adenine Nucleotide Translocator 2 (ANT2), leading to a noncompetitive inhibition of ANT activity; and (3) the release of cytochrome *c* into the cytosol (Figure 4D). The latter two effects could be caused by the depletion of PHB2 in mitochondria, which would compromise mitochondrial integrity and promote apoptosis.

Capsaicin was used in these experiments at concentrations that are much too high to be therapeutically relevant, but this work may constitute the basis for an optimization program to develop a clinically useful drug.

Sulfonyl Amidine Derivatives

Sulfonyl amidine A inhibits the osteoclastogenesis involved in bone remodeling with an IC_{50} of 1.8 μM (Lee et al., 2010c; Figure 4E). Chang et al. (2011) used affinity chromatography to identify the molecular target involved in this process and found that this compound binds to PHB1 and three other proteins. Which of these proteins is the relevant target involved in this antiresorptive activity remains to be determined.

Aftin-4

While investigating how the toxic amyloid-β42 (Aβ42) peptide is produced in Alzheimer's disease, Bettayeb et al. (2012) observed that the adenine derivative Aftin-4 promotes Aβ42 production in neurons (Figure 4F). Aftin-4 was found to interact with three proteins related to neural degeneration: PHB1, voltage-dependent anion channel 1 (VDAC1), and mitofilin.

cHOxa

The dihydrooxazole cHOxa, which displays some cytotoxicity with a GI_{50} of 7.2 μM in B16F0 cells, was shown to alkylate the Asp40 of PHB1, suggesting that it could be used as a chemical probe to analyze the structure of PHB1 (Trzeciakiewicz et al., 2011; Figure 4G).

Conclusions

The regulation by PHBs of cell survival, apoptosis, metabolism, and inflammation makes these proteins promising therapeutic targets for novel treatments for cancer and neurodegenerative, metabolic, and inflammatory diseases. Their intracellular localization and their translocation in response to proapoptotic signals differ substantially between normal and cancer cells. This divergence of behavior, which is not fully understood, may be exploited to develop therapeutic agents. The phase I trial of

adipotide for the treatment of obese patients with prostate cancer may be the first step on the road to developing PHB ligands of clinical value. The recent discovery that flavaglines target PHBs warrants further studies of this class of anticancer and cytoprotective agent. In addition to the ongoing exploration of the roles of PHBs in physiology and physiopathology, we anticipate that this field will stimulate research addressing other classes of PHB ligands with original pharmacological properties.

Over the last few years, PHBs have been found to interact with more than 60 other proteins to regulate a myriad of cellular events. There is no doubt that in the coming years, new partners and new cellular regulations will be uncovered. An important issue will be to determine when the alterations of PHB expression, subcellular localization, and signaling are involved in the etiology of not only cancers but also cardiac, neurological, inflammatory, and metabolic diseases. Translating this knowledge into clinical applications may lead to the development of new medicines and biomarkers for diagnosis and prognosis.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.chembiol.2013.02.006.

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