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Regulation of mitochondrial apoptosis by Pin1 in cancer and neurodegeneration

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ABSTRACT

Mitochondria are sensitive and efficient organelles that regulate essential biological processes including: energy metabolism, decoding and transduction of intracellular signals, and balance between cell death and survival. Of note, dysfunctions in mitochondrial physiology are a general hallmark of cancer cells, leading to transformation-related features such as altered cellular metabolism, survival under stress conditions and reduced apoptotic response to chemotherapy. Mitochondrial apoptosis is a finely regulated process that derives from activation of multiple signaling networks. A crucial biochemical requirement for transducing pro-apoptotic *stimuli* is represented by kinase-dependent phosphorylation cascades. In this context a pivotal role is played by the prolyl-isomerase Pin1, which translates Ser/Thr-Pro phosphorylation into conformational changes able to modify the activities of its substrates. In this review we will discuss the impact of Pin1 in regulating various aspects of apoptosis in different biological contexts with particular emphasis on cancer and neurodegenerative diseases.

1. Introduction

A large number of parameters essential for cell homeostasis are controlled at the level of mitochondria. These include production of energy and biosynthetic precursors, modulation of redox status, control of cytosolic calcium levels and initiation of apoptosis (Wallace, 2012). Alterations in any of these processes have dramatic consequences on normal cell homeostasis, while in tumors they foster the acquisition of aggressive phenotypes. One well-known example is the cancer-associated metabolic switch towards aerobic glycolysis known as Warburg effect (Wallace, 2005) that mainly occurs through mitochondrial reprogramming and enables cancer cells to direct glucose supplies towards biosynthesis of macromolecules, thus supporting their rapid growth. Moreover, mutations that hit mitochondrial enzymes (e.g. fumarate hydratase (FH), succinate dehydrogenase (SDH) and isocitrate dehydrogenase (IDH1)) and key components of glycolysis and Krebs cycle are a general feature of cancer cells, leading to a reduced glycolytic flux producing pyruvate and an increased flux towards biosynthetic and NADPH-producing pathways, such as Serine biosynthesis and the pentose phosphate pathway (Hitosugi et al., 2012; Locasale et al., 2011; Possemato et al., 2011; Vander Heiden et al., 2009). Another common step in the early phases of tumorigenesis is the failure of apoptotic events in response to genotoxic conditions (e.g. replication-related or oxidative stress *stimuli*). This mostly occurs as a consequence of defects within components of the mitochondrial molecular cascade, as will be discussed below.

Although many of the mitochondrial alterations that actively sustain tumor growth and progression still need to be deeply investigated, it is clear that mitochondrial dysfunctions represent one of the most important features of cancer cells, and gaining knowledge on the underlying molecular pathways may provide powerful targets for cancer therapy.

2. Mechanisms of mitochondrial apoptosis

In adult organisms, apoptotic death represents a cell-intrinsic anticancer barrier that prevents the expansion of incipient tumor cells experiencing genotoxic damage. Indeed, almost all cancer cells are defective in apoptosis (Hanahan and Weinberg, 2011) and tumorigenesis is enhanced when apoptosis is blocked (Letai et al., 2004; Vaux et al., 1988). A central molecular hub regulating apoptosis is represented by the mitochondrial permeability transition pore (mtPTP) opening in response to specific death signals, with consequent release of cytochrome c and other soluble apoptogenic mediators from the mitochondrial inter-membrane space to the cytosol, where they initiate caspase activation (Kroemer et al., 2007; Vaux, 2011). Mitochondrial outer membrane permeabilization (MOMP) is regulated at several layers, the first and most potent of them relying on the Bcl-2 family proteins. These are subdivided into three groups on the basis of both their structural homology to the prototypic member Bcl-2 and of their pro- or anti-apoptotic action (Schinzel et al., 2004; Youle and Strasser, 2008). Anti-apoptotic members (e.g. Bcl-2, Bcl-xL, Bcl-w, Mcl-1) display four Bcl-2

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Homology BH domains (Kvansakul et al., 2008), while pro-apoptotic Bax-like proteins (e.g. Bax, Bak) have three of them (BH1, BH2, BH3) and the pro-apoptotic BH3-only proteins (e.g., Bid, Bim, Noxa and Puma) possess the sole BH3 domain and are master regulators of the entire process (Giam et al., 2008). To induce apoptosis, the effectors Bax-like proteins are activated by conformational changes and homo-oligomerization, which in turn lead to their translocation or repositioning into the outer mitochondrial membrane (OMM) to generate pores that release pro-apoptotic factors from the mitochondrial intramembranous space. The BH3-only class of proteins serves the role of favoring such activation, either by stimulating the oligomerization of Bak and Bax (tBid and Bim proteins) or inhibiting the binding between anti-apoptotic proteins and the effectors of apoptosis (Puma, Noxa and Bad proteins)(Kroemer et al., 2007). There are several examples of altered expression and function of Bcl-2-family members leading to apoptosis resistance in cancer. The prototypic family member, i.e. Bcl-2, was found involved in t(14;18) chromosomal translocations in non-Hodgkin's lymphomas, causing its deregulated expression (Rampino et al., 1997; Tsujimoto et al., 1985), while homozygous deletions or inactivating mutations of the Bax gene have been found in several tumor types (Rampino et al., 1997).

Another layer of control on mitochondrial apoptosis is exerted by the remodeling of mitochondrial shape through fusion and fission events, which presides over the swift protein efflux from mitochondria during MOMP. Some proteins involved in mitochondrial dynamics, such as Drp-1 and the mitochondrial fusion protein Mfn-2, are co-localized with Bax at apoptotic mitochondria (Karbowski et al., 2002) and stabilization of Drp-1 in mitochondrial membranes is Bax/Bak dependent (Wasiak et al., 2007). These studies imply that there is a close coordination between MOMP and mitochondrial dynamics, although the molecular mechanisms remain unknown.

Finally, a crucial role in apoptosis induction is played by intracellular calcium trafficking. The endoplasmic reticulum (ER) is the major storage of Ca^{2+} ions. Under specific physiological or pathological *stimuli* it can release Ca^{2+}, which is immediately taken by the mitochondria. This calcium transfer between the ER and mitochondria regulates several biological processes among which apoptosis has recently emerged. In particular, it has been demonstrated that calcium overload leads to swelling and fragmentation of mitochondria with consequent release of cytochrome c into the cytoplasm and apoptotic cell death induction (Pinton et al., 2008).

3. The many roles of the prolyl-isomerase Pin1 in mitochondrial apoptosis

MOMP activation in response to intracellular *stimuli* and the subsequent release of apoptogenic factors represent a point of no return in the apoptosis pathway. Once MOMP has occurred, cells are unable to recover due to defective mitochondrial function (Chipuk et al., 2005; Lartigue et al., 2009). For this reason, MOMP is subjected to tight control, both at the transcriptional level and via direct protein interactions. One of the most important and universal mechanisms regulating transition states in the cell is represented by the reversible phosphorylation of proteins (Liou et al., 2011) downstream to activated signaling pathways. In particular, phosphorylation of certain Serine or Threonine residues preceding a Proline (pSer/Thr-Pro) represents a key event for many proteins, that in this way are subjected to a further, sophisticated layer of control based on post-phosphorylation conformational changes, on which their full activation relies. This crucial function is operated by a unique enzyme, the phosphorylation-specific peptidyl-prolyl cis/trans isomerase Pin1, able to recognize pSer/Thr-Pro motifs and catalyze the cis-trans isomerization of the intervening peptide bond with consequent alteration of important properties of its targets such as the phosphorylation status, interaction profile and stability, among others. Pin1 thus governs a variety of cellular processes including cell cycle, transcription and splicing, RNA editing, DNA damage and oxidative stress responses, germ cell development, stem cell self renewal/expansion and neuronal survival (Brenkman et al., 2008; Lu and Zhou, 2007; Pinton et al., 2007; Rustighi et al., 2014a; Yeh and Means, 2007).

4. Pin1: structure, activity and regulation

The human Pin1 gene maps to chromosome 19p13 and encodes a protein of 163 amino acids with a mass of 18 kDa. From a functional point of view, Pin1 is composed of two domains: an amino terminal WW domain (amino acids 1–39) and a carboxy terminal PPIase domain (amino acids 45–163), which are separated by a short flexible linker region. The N-terminal WW region is characterized by two conserved tryptophan residues and mediates the interaction with the substrates on pSer/Thr-Pro sites, which cannot be bound by any other isomerase (Ranganathan et al., 1997; Yaffe et al., 1997). As a consequence of the interaction, the WW domain targets the Pin1 catalytic domain close to its substrates, so that the PPIase domain can isomerizes specific pSer/Thr-Pro motifs and induce conformational changes (Lu and Zhou, 2007).

Pin1 knock-out mice display a panel of developmental and age-related phenotypes that can be reconducted to specific functions of Pin1 observed *in vitro*. In particular, mice lacking Pin1 have reduced body weight compared to their wild-type littermates, germ cell deficiency, abnormal neuronal differentiation and impaired motor activity, alteration of breast stem cell compartment with consequent impairment of mammary gland development, in addition to retinal degeneration and testicular atrophy (Atchison and Means, 2003; Liou et al., 2002; Nakamura et al., 2012; Rustighi et al., 2014a; T.H. Lee et al., 2011b). All these defects prove that Pin1 plays a pleiotropic role as a master regulator of diverse signaling pathways.

In addition, more defects including those related to Pin1 function in apoptosis might be discovered in these mice following particular challenging *stimuli*, that would unveil a role for Pin1 in disease-related and acute stress signaling, as shown for example in diabetic mice (Paneni et al., 2014).

Several physiological and pathological conditions regulate Pin1 levels and activity. Pin1 expression is profoundly linked to cell proliferation status, and results aberrantly increased in several human cancers being induced by E2F in response to growth factor stimulation and oncogene activation (such as Notch), while being repressed by the tumor suppressor BRCA1 (MacLachlan et al., 2000; Rustighi et al., 2009; Ryo et al., 2002). In addition, both regulation of Pin1 subcellular localization (Magli et al., 2010) and post-translational modifications impact on cellular functions and activity of Pin1, respectively. Phosphorylation of Ser 16 within the WW domain is regulated during the cell cycle and alters its ability to recognize its protein targets (Lu et al., 2002). Other phosphorylations, such as that catalyzed by the tumor suppressor kinase DAPK1 on the Pin1 isomerase domain, are able to inhibit its catalytic activity. Polo-like-kinase-1 (PLK1) has been shown to phosphorylate Pin1 on Ser65, thus reducing its ubiquitination and proteasomal degradation (Eckerdt et al., 2005). Finally, inhibitory oxidative modification of Pin1 occurs in Alzheimer's Disease (AD) brains (Sultana et al., 2006). All these layers of regulation may disclose alternative ways to manipulate Pin1 activity for therapeutical purposes.

5. Pin1 is a key regulator of apoptotic events in cancer

In recent years, accumulating evidence has pointed out a crucial role for Pin1 in the activation of several cellular signaling pathways, by acting as a context-dependent signal transducer based on specific genetic and environmental cues. Interestingly, altered Pin1 function has been observed both in cancer and neurodegenerative diseases. Pin1 overexpression is frequently found in most common human cancers including breast, prostate, lung, colon, esophageal cancer, oral squamous cell carcinoma, glioblastoma and ovary, cervical and melanoma tumors (Atkinson et al., 2009; Bao et al., 2004; Jin et al., 2011; Miyashita et al., 2003) and is correlated with poor clinical outcome (Ayala et al., 2003;