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Previews

Prohibitin(g) Cancer: Aurilide and Killing by Opa1-Dependent Cristae Remodeling

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Proapoptotic drugs targeting the mitochondrial Bcl-2 rheostat of apoptosis are tools to selectively kill cancer cells. Sato et al. (2011) expand the available toolkit by identifying the target of the cytotoxic natural product aurilide in the prohibitin Opa1-dependent apoptotic cristae remodeling.

Programmed cell death and its morphologic manifestation of apoptosis is a conserved pathway that, in its basic tenets, appears operative in all metazoans. During embryonic development, cell death is essential for successful organogenesis and the crafting of complex multicellular tissues (Wasilewski and Scorrano, 2009). Apoptosis also operates in adult organisms to maintain normal cellular homeostasis, and resistance to apoptosis is a hallmark of cancer, often contributing to chemoresistance. Several key apoptotic pathways are altered in cancer, including the loss of function mutations in the p53 tumor suppressor gene, as well as the overexpression of antiapoptotic proteins such as the mitochondrial antioncogenes Bcl-2 and Bcl-X_L (Hanahan and Weinberg, 2000). Mitochondria are key organelles in apoptosis, since they amplify prodeath stimuli by releasing into the cytoplasm cytochrome *c* and other protein cofactors that are required for the activation of the caspases that execute the demise of the cell. The permeabilization of mitochondria during apoptosis is controlled by members of the Bcl-2 family and is accompanied by morphological and ultrastructural changes (the so-called "cristae remodeling") that allow the complete release of cytochrome *c* (Wasilewski and Scorrano, 2009). Defective apoptosis allows cancer cells to extend their lifespan, to acquire further genetic mutations, to grow under unfavorable conditions, and even to support tumor angiogenesis. Since survival of cancer cells depends on these changes in apoptosis, novel "selective" anticancer drugs are being produced that could, in principle, spare normal cells that are less dependent on these alterations (Hanahan and Weinberg, 2000). For example, several drugs that target the Bcl-2 proteins are already in clinical trials (Zhang et al., 2007). In this issue, Sato et al. (2011) extend the molecular pathways targeted by proapoptotic drugs to the ultrastructural changes occurring to mitochondria during apoptosis.

Aurilide is a small cyclodepsipeptide derived from *Dolabella auricularia* that exerts potent cytotoxic effect. This molecule induces apoptosis in human cancer cells at low concentrations (Suenaga et al., 2008), but its molecular targets were unknown. Sato et al. (2011) demonstrate that aurilide selectively binds to prohibitin (Phb) 1, thereby identifying for the first time a molecule that induces apoptosis through the pathway of mitochondrial cristae remodeling controlled by Phb and by the dynamin-like GTPase optic atrophy 1 (Opa1). Affinity matrix chromatography followed by mass spectrometry of the identified peptides revealed Phb1 as a unique aurilide-binding protein, whereas its homolog Phb2 had no detectable affinity for the biomolecule. Phb1 and Phb2 are ubiquitously expressed membrane proteins that are highly conserved throughout eukaryotes. They are involved in several cell processes and they play a crucial role in sustaining cancer cell proliferation and adhesion (Sievers et al., 2010). Phb1 and Phb2 form large assemblies in the inner mitochondrial membrane where they regulate the processing of Opa1 (Merkwirth et al., 2008). Opa1 has genetically distinguishable functions in mitochondrial fusion and in apoptosis by regulating the process of cristae remodeling. Remodeling of the mitochondrial cristae is required to mobilize the bulk of cytochrome *c* from the cristae space to the intermembrane space, where it can be released across the outer mitochondrial membrane. Enforced expression of Opa1 blocks cristae remodeling and delays apoptosis, substantiating a role for cristae remodeling in the control of cell death (Frezza et al., 2006).

Genetic deletion of Phb results in aberrant cristae morphogenesis, impaired cell proliferation, and increased apoptosis, dependent on the increased proteolytic processing of Opa1 (Merkwirth et al., 2008). Phb1 interacts with spastic paraplegia 7 (SPG7), a component of human m-AAA protease complex involved in Opa1 processing (Wasilewski and Scorrano, 2009). Aurilide treatment resulted in Phb1-SPG7 complex disruption, suggesting that aurilide could interfere with normal Opa1 processing. Furthermore, aurilide accumulated in mitochondria and induced fragmentation of the organelle, similar to what is observed in cells lacking Phb. Accordingly, aurilide accelerated the processing of Opa1, resulting in a greater conversion of long into short isoforms. However, this does not confirm that the killing mechanism depends on Phb and Opa1. This was elegantly tested by Sato et al. (2011) by Phb1 silencing and overexpression experiments that confirmed that aurilide depended on the Phb complex. Finally, cells expressing a mutant of Opa1 that is resistant to proteolysis were protected from aurilide, further substantiating how stability of Opa1 is important for the mechanism by which this molecule induced cell death. Indeed, a high molecular weight oligomer composed of both short and long forms of Opa1 determines the resistance of

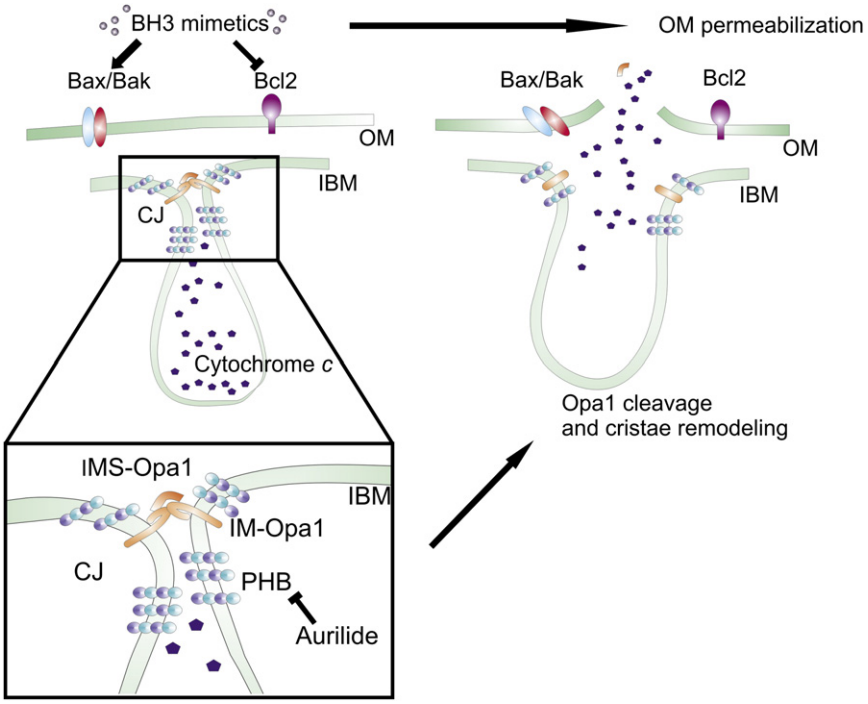

Figure 1. Molecular Mechanisms of Action of Proapoptotic Mitochondrial Drugs: BH3 Mimetics and Aurilide

While BH3 mimetics lead to outer membrane permeabilization by acting on the Bcl-2 rheostat, aurilide shows a new mechanism by acting on the Phb/Opa1-dependent cristae remodeling pathway. Key molecular players are defined in the legend. OM, outer membrane; IBM, inner boundary membrane; CJ, cristae junction; IMS-Opa1, soluble Opa1; IM-Opa1, membrane integral Opa1.

mitochondria to release cytochrome *c*. Aurilide is in this respect the first molecule shown to specifically activate the Opa1-dependent branch of apoptosis by selectively inhibiting Phb1, ultimately leading to the processing of Opa1, the destabilization of its oligomers, and the enhanced release of cytochrome *c*. It is still unclear how the disruption of normal Opa1 function can lead to permeabilization of the outer membrane. In this respect, it is conceivable that aurilide has additional mitochondrial targets that result in the activation of the multidomain proapoptotics, or that destabilization of cristae structure is somehow transmitted to the outer membrane. Future work will elucidate the fine mechanism, and analogs of aurilide with lower mitochondria-permeabilizing effects might be of considerable value in combined chemotherapy with Bcl-2 inhibitors, for example.

The work of Sato et al. (2011) opens new avenues in the quest for targeted proapoptotic molecules that act at the mitochondrial level and offers a proof of the principle that mitochondrial shape changes can be targeted to induce apoptosis in cancer cells, as they have been exploited to conversely reduce damage in neurodegenerative (Costa et al., 2010) and ischemic diseases (Wang et al., 2010). In this respect, it is interesting that in earlier studies aurilide has been reported to be exquisitely active against cells derived from prostate cancer and leukemias (Han et al., 2006), suggesting an exploration of whether levels of Phb or Opa1 are upregulated in these specific cancers.

In conclusion, this elegant work represents the successful identification of a drug that induces apoptosis by interfering with the normal mechanisms that govern the morphogenesis of mitochondria, and by destabilizing the cristae-shaping protein Opa1 (Figure 1). Future work will definitely increase the molecular toolkit that activates mitochondrial apoptosis from the inner membrane.

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