In the new study, Hemsworth *et al.*[4] noticed that some LPMOs are multimodular proteins carrying a small conserved domain of unknown function called X278. A wide search using a 'module walking' approach looking for proteins containing the X278 domain returned approximately 450 sequences of a possible new enzyme family in fungal genomes. The sequences have a signal peptide followed by a conserved histidine, indicating that they are extracellular proteins and that the N-terminal histidine can coordinate a metal ion like previously known LPMOs. One of these proteins in the new candidate family from a filamentous fungus (familiar to some because it is the lead character in the manga comic *Moyashimon*) was characterized in detail: Ao(AA11) from *Aspergillus oryzae* (*koji* mold) was demonstrated by MALDI-TOF analysis to have oxidative chitinolytic activity yielding aldonic acid chitooligosaccharides. X-ray crystallography, isothermal titration calorimetry and EPR spectroscopy indicated that the AA11 enzyme has structural and spectroscopic characteristics somewhat in between those of the AA9 and AA10 families. The overall fold and metal coordination were basically similar to the known LPMOs, but one interesting feature of the new family is a slightly convex surface where the copper active site sits.

These results establish the structural and biochemical foundations of a third family of LPMOs. It is exciting that, after a long delay following the initial reports on the importance of oxidative decomposition of biomass from the 1970s[9], we are finally gaining momentum in identifying and characterizing these enzymes. Indeed, this initial report of the AA11 family raises questions about these newcomers and the LPMOs more generally: Are the structural commonalities of current LMPO families (the β-sandwich fold and the copper coordination involving the N-terminal histidine) indispensable for the oxidative chain-cleavage functionality? Or does the discovery of an LMPO with a different structural scaffold await our further exploration? What is the electron donor of LPMOs in the natural environment where they work? Some reports indicate that in nature AA9 may receive electrons from cellobiose dehydrogenase[3,10], which previously has been thought to provide electrons for ferric ion, but this remains to be tested carefully. Which route do the electrons take into the copper active center? A line of conserved aromatic residues in the center of the β-sandwich fold has been implicated in this role, but it is still to be verified, along with identification of the binding site for electron donors. Finally, how does the mono-oxygenase reaction take place at the active site of these unusual enzymes? The copper active site of LPMOs has a notable similarity to that of copper methane mono-oxygenase, which can act on the highly oxidation-resistant substrate methane[2] and so may provide some starting points for further analysis. No matter how these enzymes catalyze their reactions, the discovery of a new LPMO family is truly good news for people who want to achieve effective biomass decomposition, as they both provide a new enzymatic tool and point to further possible discoveries from the abundant and growing microbial genome information.

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Competing financial interests

The author declares no competing financial interests.

CANCER THERAPY

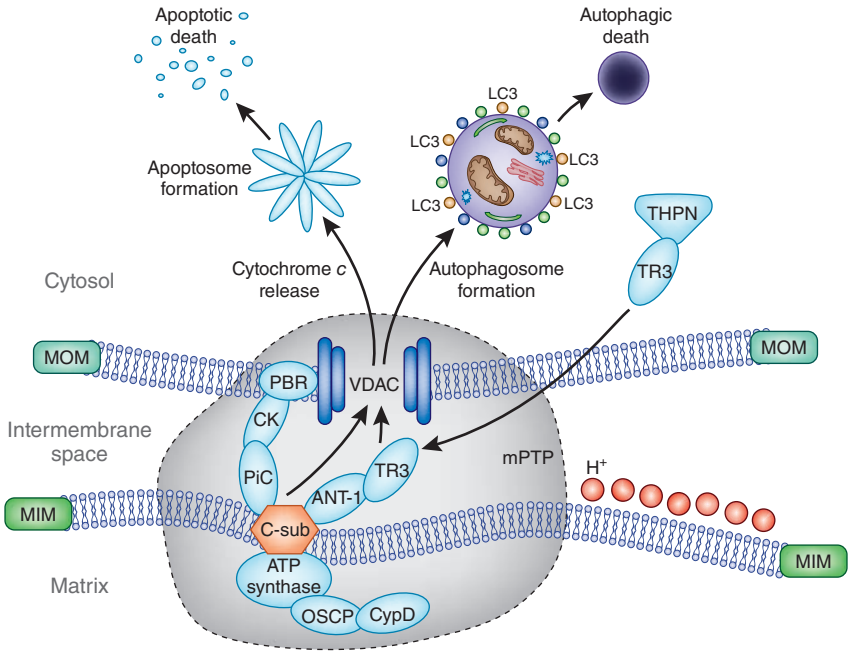
Altering mitochondrial properties

The mitochondrial permeability transition pore (mPTP) is a multiprotein complex that regulates cell death in multiple pathological conditions. The discovery of new critical components of the mPTP and the identification of anticancer drugs acting on mPTP opens new frontiers for mitochondrial medicine.

Paolo Pinton & Guido Kroemer

In the multistep pathway of neoplastic transformation, cancer cells accumulate mutations, allowing them to proliferate and survive in a deregulated manner as they escape elimination by the immune system[1]. The illegitimate survival of cancer cells can be caused by genetically or epigenetically determined mechanisms affecting the molecular machinery of apoptosis, and such changes simultaneously determine resistance to radio- or chemotherapy[2]. In this issue, Wang *et al.*[3] describe a new signaling pathway that controls the mitochondrial activity of the orphan nuclear receptor TR3 (also known as Nur77 or NGFI-B) (**Fig. 1**) as they elaborate a new pharmacological strategy for cell death induction in a particularly malignant and apoptosis-resistant skin cancer known as melanoma. For this, the authors identified 1-(3,4,5-trihydroxyphenyl)nonan-1-one (THPN) as a drug that specifically targets the TR3 protein and kills mouse melanoma cells[3].

Mitochondria, traditionally viewed as the powerhouses that supply energy to cells, are also considered vessels filled with self-destructive weaponry that can be unleashed to promote the apoptotic signaling cascade[4]. Lethal stimuli cause the release of several proapoptotic mediators from the mitochondrial intermembrane space into the cytosol. The interaction with cytosolic proteins of one of these mediators, cytochrome *c*, results in assembly of a complex (apoptosome) that recruits and activates caspases, a class of proteases that can participate in the apoptotic dismantling of cells[5]. The molecular mechanism of cytochrome *c* release is still unclear but most likely requires the activity of the mPTP, a large-conductance channel



Katie Vicari

Figure 1 | Putative mechanisms through which THPN-TR3 complexes induce autophagic cell death. Upon interaction of THPN to the TR3 protein, TR3 translocates crosses the mitochondrial outer membrane (MOM) to interact with ANT-1 at the mitochondrial inner membrane (MIM). This interaction triggers a molecular cascade that may involve other proteins that contribute to the formation of the mPTP such as VDAC1, CypD, ANT-1, the C subunit of the mitochondrial ATP synthase (C-sub), the mitochondrial inorganic phosphate carrier (PiC), creatine kinase (CK), the oligomycin sensitivity–conferring protein (OSCP) and the peripheral benzodiazepine receptor (PBR). The ultimate outcome of this process is the loss of the inner mitochondrial transmembrane potential and MOM permeabilization, resulting into either apoptosis or autophagy. Autophagic membranes are decorated by the microtubule-associated protein 1A/1B-light chain 3 (LC3) protein.

located in mitochondrial membranes. Important components of the mPTP include cyclophilin D (CypD) in the mitochondrial matrix, adenine nucleotide translocase-1 (ANT-1) and the C subunit of the mitochondrial ATP synthase in the inner membrane and voltage-dependent anion channel-1 (VDAC1) in the outer mitochondrial membrane[5,6]. In response to proapoptotic stimuli, such as ROS and Ca^{2+} overload, the mPTP assumes a high-conductance state that ultimately results in the dissipation of the mitochondrial transmembrane swelling ($\Delta\psi_m$) and concomitant large-scale alterations of organelle morphology that facilitates the release of cytochrome *c* into the cytosol[5].

It has been previously known that, under stress conditions, TR3 translocates from the nucleus to mitochondria[7]. However, there has been no evidence that TR3 may be targeted directly by small molecules. Using a combination of different techniques including crystallography, cell biology and genetic approaches, Wang *et al.*[3] present evidence that THPN can directly interact with the ligand-binding domain of TR3. The binding of TR3 to THPN then promotes a conformational change in the THPN protein that, within the cellular context, favors its translocation to the mitochondria through a complex pathway. TR3 first docks to the proapoptotic protein NIX on the mitochondrial surface and then crosses (at least) the outer mitochondrial membrane through the interaction with proteins of the import machinery and ultimately triggers ANT-1–VDAC1-dependent $\Delta\psi_m$ dissipation[3]. Surprisingly, this process does not result in apoptosis but rather triggers autophagy.

Autophagy, the housekeeping mechanism for recycling cellular components is now recognized as a response to various stress conditions, with two distinct, antonymic outcomes: (i) adaptation of cells to hostile conditions due to the mobilization of energy reserves and the recycling of damaged organelles, including permeabilized mitochondria, and (ii) autophagic cell death (or 'autosis') due to the digestion of essential parts of the cell[8]. Although this has not been formally demonstrated, it seems plausible that quantitative differences in autophagy dictate its role in adaptation versus death.

Importantly, apoptosis and autophagy may intersect at the mitochondrial level. Ca^{2+} is an important activator of both processes, acting on distinct molecular targets[9] including the mPTP, which may induce both apoptosis and autophagy[10]. The current work by Wang and colleagues[3] reinforces this concept and provides evidence that massive pharmacological activation of the mPTP can trigger cell death via a pathway that involves autophagy, at least in apoptosis-resistant melanoma cells. Indeed, knockdown of distinct mPTP components (such as ANT-1, CypD and VDAC1) or of several essential autophagy-related proteins (such as ATG5 and ATG7) abolished the induction of melanoma cell death by THPN[3].

It will be interesting to follow up this work to understand though which precise mechanisms TR3 triggers mPTP opening and how mPTP opening then activates the autophagic removal of mitochondria, ultimately resulting in cell death. Moreover, it will be important to learn whether and, if so, why THPN can stimulate this pathway specifically in melanoma but not in other cell types. Understanding these mechanisms will be essential for the further development of THPN as an anticancer agent. ■

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