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Salvatore Nesci

Department of Veterinary Medical Sciences, University of Bologna

Via Tolara di Sopra, 50 – 40064 Ozzano Emilia, Bologna, Italy.

*Correspondence: salvatore.nesci@unibo.it

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Abstract

The mitochondrial ATP synthase synthesizes ATP for cellular function but under various conditions including ischemia it hydrolyzes ATP, primarily to re-energize the mitochondria. ATPIF1 inhibits ATP synthase hydrolysis of ATP. Kaelin and colleagues recently demonstrated that GPR35 is involved in this process. This finding provides an additional framework for novel discovery of potentially therapeutic molecules against ischemia/reperfusion injury.

Main Text

The maintenance of mitochondrial bioenergetics for ATP production, ions and solute homeostasis during ischemia is important for cardioprotection. During ischemia/reperfusion (I/R), extensive generation of **Reactive Oxygen Species** (ROS) production triggers the **mitochondrial Permeability Transition Pore** (mPTP) which leads to impairment of mitochondrial bioenergetics and swelling, rupture of inner mitochondrial membranes (IMM) and, finally, **regulated cell death** (RCD) [1,2]. There has been strong interest in the regulation mechanism of ATP production by cardioprotective compounds/drugs during I/R injury since homeostatic ATP level is vital for myocardial function. Orphan **G protein-coupled receptor 35** (GPR35) is associated with GPR35-mediated cardioprotection, although the mechanism is unclear. A model of the human interactome in HCT116 cells reported a 97% of high-confidence probability of interaction between GPR35 and the mitochondrial subunit **ATP synthase Inhibitory Factor 1** (ATPIF1) [3]. ATPIF1 inhibits the ATP hydrolysis by the ATP synthase but not ATP synthesis. Actually, in cardiovascular diseases, the role of ATP synthase in ATP production is well known but not of GPR35. **ATP synthase** is a multimeric enzyme composed of two domains named F_O and F_1 that are kept together by a central stalk (the rotor) and a peripheral stalk (the stator). The F_O domain is hydrophobic and membrane-embedded. It transduces the transmembrane energy electrochemical gradient of proton (H^+), generated by respiration, into the spherical catalytic globular

assembly – the place of ATP synthesis or hydrolysis reactions – of the F_1 domain by a rotary action. The H^+ flux, passing through the two half-channels formed at the interface between *a* subunit and the *c*-ring structure of F_O domain, powers the torsion responsible for the phosphorylation of ADP to ATP. Conversely, in reverse function, ATP hydrolysis drives the torsion in opposite direction and the enzyme energizes the IMM working as an H^+ pump [4]. Studies to elucidate how GPR35 may be regulating ATP production and cardioprotection would advance the field for the development of novel potential therapeutics.

Interestingly, Kaelin's group demonstrated that GPR35 activated by agonists could relay a protective signal to the ischemic heart [5]. The experimental results of Wyant and colleagues obtained by confocal microscopy analysis showed that GPR35 colocalized with mitochondrial proteins such as ATP synthase subunits and citrate synthase. Pamoic acid, zaprinast, and lodoxamde are well-characterized ligands of GPR35. They activate GPR35 by binding to a narrow space between transmembrane domains III and VII of GPR35 (Figure 1A), which provides the principal site for ionic interaction with ligands. **Kynurenic acid** (kyna) is the main bioactive metabolite of tryptophan degradation. Kaelin's group showed that kyna exhibited agonist characteristics and activates GPR35 by binding to the binding site of known GPR35 activators. Using isolated mitochondria obtained from 293FT cells stably expressing GPR35, they showed GPR35 association with the OMM in kyna-pretreated cells [5]. They demonstrated that kyna binding to GPR35 is required for kyna-induced ischemic protection in human and murine cardiomyocytes via the promotion of ATP synthase supramolecular organization and anti-ischemic mechanisms of ATP conservation [5].

Interesting, the results of Kaelin's group are linked to the biological role of ATP synthase in mitochondrial morphology and to the bifunctional mechanism of synthesis or hydrolysis of ATP regulated by ATPIF1, which determines cell fate during ischemia [2]. The monomeric form of ATP synthase can dimerize at the apex of the ***cristae*** and form long row-like oligomers. The conformation of the ATP synthase dimer at the edge of *cristae* adopts the classical V-shape, whereas the supramolecular assembly in rows of dimers along the highly curved edges of *cristae* adopt a tetrameric structure of dimers with H-shape [6] (Figure 1B). These oligomers influence the tightly curved *cristae* ridges and have been shown to be required for maintaining mitochondrial morphology and preventing mPTP formation [7,8]. At around neutral pH, active ATPIF1 dimer forms α-helices antiparallel **coiled-coil** interactions at the C-terminal regions imposing its two N-terminal inhibitory domains facing opposite sites, thereby simultaneously interacting with two adjacent F_1 domains of two different ATP synthase dimers (Figure 1C). The ATPIF1 dimeric form fulfils a "ratchet" role in blocking only reverse rotor rotation of ATP synthase driven by ATP hydrolysis. This molecular mechanism regulated by ATPIF1 controls the ATP dissipation in mitochondria. Also, ATPIF1 joins two opposite protomers of ATP synthase of two adjacent dimers to improve the stability of the ATP synthase tetramer [6] (Figure 1B). In addition to the pH-dependent regulation allowed by the protonation state of histidine residues, ATPIF1 is regulated by phosphorylation/dephosphorylation at the Ser39 [9]. Deactivation of ATPIF1 by phosphorylation allows the ATP hydrolytic activity of ATP synthase. On the contrary, ATPIF1 activation by dephosphorylation of Ser39 blocks ATPase activity of ATP synthase (Figure 1C). This study by Wyant and colleagues established at the molecular level the indirect action of GPR35 on ATPIF1's effect on ATP synthase supramolecular assembly and ATP hydrolysis. They showed that kyna mediated GPR35 internalization and OMM association and regulated the interaction between ATP synthase and ATPIF1 by hindering **posttranslational modification** (PTM). The dephosphorylated ATPIF1 by interacting with the ATP synthase dimers inhibits ATP hydrolysis and preserves the ATP pool and cell viability during ischemia [5]. They explained this unknown molecular mechanism and concluded that the mitochondrial GPR35 signalling negatively affects the cAMP production by adenylyl cyclase (AC). Accordingly, GPR35 indirectly blocks the cAMP-dependent **protein kinase A** (PKA) phosphorylation processes on ATPIF1. This finding is important because an active ATPIF1 has the physiological role of inhibiting ATP hydrolysis, but not ATP synthesis which is sustained by the forward rotation of the ATP synthase rotor. The discovery establishes the basis for the pharmacological use of kyna-mediated GPR35 activation in preventing mitochondrial dysfunctions caused by ATP synthase which works as an energy dissipator during ischemia.

Cellular ATP depletion caused by ischemia is linked to mitochondrial ROS production and increased Ca^{2+} concentration, which are precursor events of mPTP formation and RCD [2,8]. During mPTP, Ca^{2+} accumulation in the mitochondria activates AC, the main source of cAMP; which triggers ATPIF1 phosphorylation and inactivation [9]. GPR35, acting through ATPIF1 as an ATP regulator, aims at the conservation of ATP levels and prevents, during ischemia, the transformation of mitochondria from ATP producers to ATP consumers. Therefore, inhibition of ATP hydrolytic activity of ATP synthase by ATPIF1 can prevent Ca^{2+}-induced conformational changes in the ATP synthase monomer responsible for ATP synthase dissociation from dimers to monomers allowing the mPTP formation [4,10].

In addition to this, kyna-mediated GPR35 activation can have pleiotropic effects on impaired mitochondria during ischemia. GPR35 could be protective at different points after ischemia hampering overload of the electron transport chain and related ROS production that consequently induces mPTP opening.

In summary, the work by Wyant *et al*. uncovers the GPR35-mitochondria axis as a promising new biological target for counteracting cardiovascular dysfunction during mPTP and ischemia. The block of mPTP opening [7], which promotes RCD, could be controlled with ATP hydrolase activity inhibition. The discovery of pharmacological strategies to activate GPR35 and ensure that ATPIF1 binds to the ATP synthase so as to prevent ATP depletion during ischemia may address drug design and improve the therapeutic options to fight I/R injury and ischemia.

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Declaration of Interest

The author declares no conflicts of interests

Glossary

G protein-coupled receptor 35: in humans, it is encoded by the GPR35 gene and is still considered an orphan cell surface receptor.

Reactive Oxygen Species: these are highly reactive chemicals formed from O_2 generated during impaired mitochondrial oxidative metabolism.

mitochondrial Permeability Transition Pore: it is a high conductance channel located in the IMM that mediates the Ca^{2+}-dependent permeability increase of the IMM to ions and solutes.

regulated cell death: it is a ubiquitous process in living organisms that depends on the activation of a coded genetic program and can be genetically and pharmacologically modulated.

post-translational modification: it is a chemical modification by the covalent addition of functional group(s) or protein(s), proteolytic cleavage of the regulatory subunit(s), or degradation of the entire protein(s). These modifications include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, and ac(et)ylation.

ATP synthase Inhibitory Factor 1: it is a protein encoded in the ATPIF1 nuclear gene and is the physiological inhibitor of the mitochondrial ATP synthase.

kynurenic acid: it is a product of the physiological metabolism of L-tryptophan product, synthesized from one branch of the kynurenine pathway.

ATP synthase: it is a bifunctional nanoscale machine with a rotary mechanism of electrochemical transducer used during the oxidative phosphorylation to produce ATP from ADP and Pi, whereas working in reverse as a proton pump hydrolyzes ATP and energizes the membrane.

cristae: these are folds of the IMM that protrude into the mitochondrial matrix with a lamellar or tubular shape that depends on mitochondrial dynamics and biogenesis.

coiled-coil: it is a structural motif in proteins in which alpha helices in the secondary structure of proteins are twisted together like the threads of a rope.

protein kinase A: it is a protein activated by cyclic AMP that targets serine and/or threonine residues by covalent binding of a phosphate group obtained from ATP hydrolysis.

Figure legends

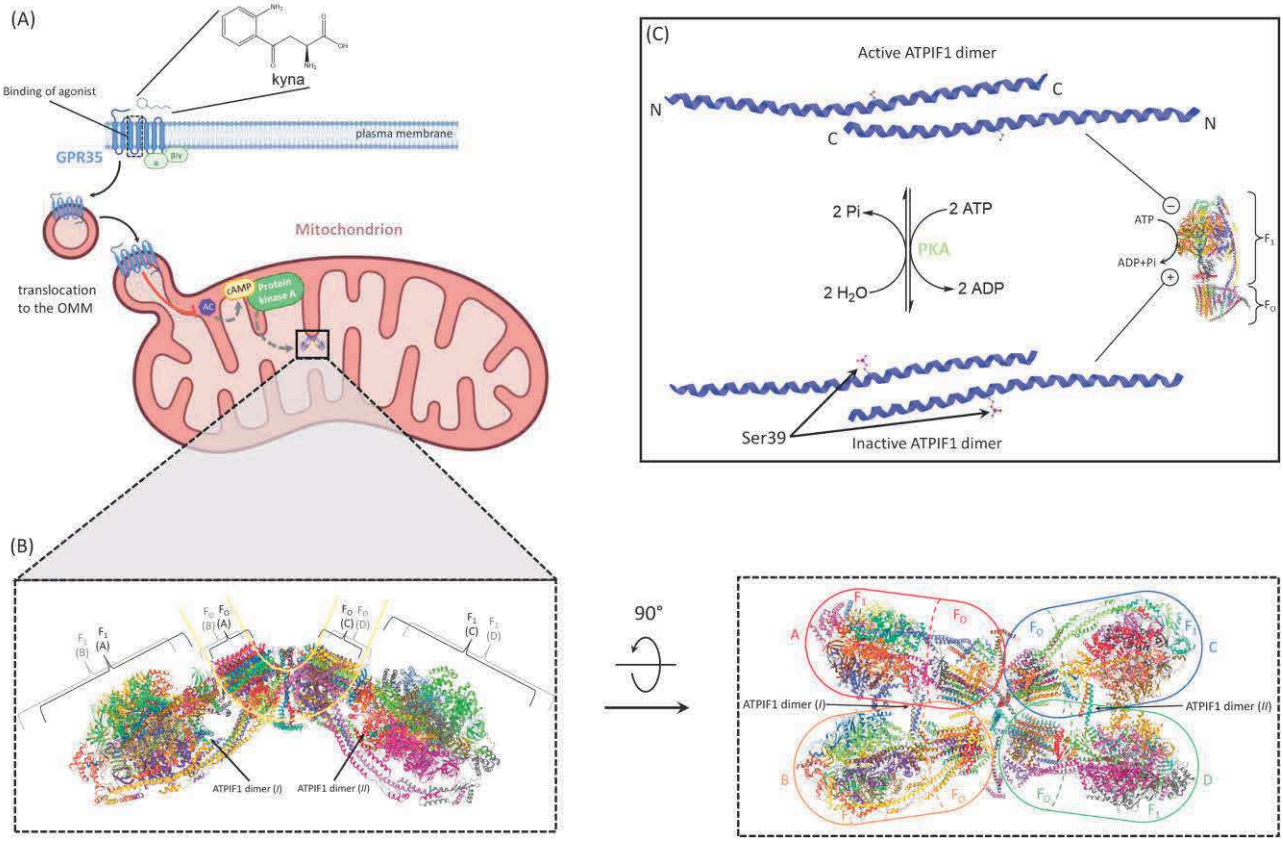

Figure 1. Signal transduction of GPR35 by kyna-mediated regulated interaction between ATPIF1 and ATP synthase. A) Kyna binds GPR35 on the plasma membrane and induces receptor translocation to the OMM. Translocated activated GPR35 on OMM inhibits the mitochondrial AC by blocking the cAMP production necessary for PKA activation. PKA can induce PTM of ATPIF1 dimer by phosphorylation of Ser residue. ATPIF1 in dimeric form can assemble with the supramolecular organization of ATP synthase supercomplex at the apex of the *crista*. B) ATP synthase tetramer representation with ATPIF1 dimer linked to two ATP synthase monomers of two adjacent dimers is viewed laterally (upper panel) and from the matrix (lower panel). C) Active and inactive state of ATPIF1 dimer. Dephosphorylated state of ATPIF1 is the active form that inhibits the ATP synthase reverse function under ischemic conditions by preventing ATP hydrolysis. On the contrary, the inactive form of ATPIF1 dimer obtained after PKA-dependent phosphorylation cannot inhibit ATP synthase-dependent ATP depletion. N-terminal, N; C-terminal, C.The ATP synthase tetramer and ATPIF1 dimer are drawn as ribbon representations obtained from modified PDB ID codes: 6J5K and 1GMJ, respectively. Figure created with BioRender.com and Chem3D.