

Moving nuclear receptor Nur77 to damaged mitochondria for clearance by mitophagy

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ABSTRACT

Selective clearance of damaged mitochondria can reverse pathological status in chronic inflammatory diseases. We recently identified a critical role of nuclear receptor Nur77 and celastrol in priming inflamed mitochondria for autophagy through its mitochondrial targeting and interaction with tumor necrosis factor receptor-associated factor 2 (TRAF2) and the autophagic adaptor p62/SQSTM1.

Abbreviations: NGFI-B, nerve growth factor IB; PGC-1 α , peroxisome proliferator-activated receptor γ coactivator-1 α ; TNF α , tumor necrosis factor- α ; TRAF2, tumor necrosis factor receptor-associated factor 2

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Mitochondrion, an evolutionally preserved organelle, plays a central role in many biologic processes including energy production, cell death, immunity, and inflammation.¹ The organelle is susceptible to damage from inflammatory mediators, a process that is implicated in the development of numerous inflammatory diseases, cancer, and aging. Clearance of damaged mitochondria through autophagy (mitophagy) has been identified as a plausible mechanism for mitochondrial quality control, representing an effective approach to reverse pathological states in chronic inflammatory diseases.

Nur77 (NR4A1, best known as Nur77, TR3, and NGFI-B) is an orphan member of the nuclear receptor superfamily and an immediate early response gene, which plays an integral role in a plethora of cellular processes in response to diverse intracellular and extracellular stimuli such as mitogens, cytokine, stress, and metabolic and apoptotic signals.²⁻⁴ Genetic studies have revealed a critical role of Nur77 in the onset and progression of cancer, and metabolic and inflammatory diseases. Nuclear receptors are traditionally considered to act in the nucleus as transcription factors to modulate gene expression. We previously reported that Nur77, in response to certain apoptotic stimuli, could translocate from the nucleus to mitochondria to induce cytochrome *c* release and apoptosis of cancer cells,^{3,4} revealing a nongenomic action of a nuclear receptor at mitochondria. Recently, we discovered an additional role of Nur77 at mitochondria involving clearance of damaged mitochondria through autophagy.⁵

Our screening effort identified celastrol as a potent binder of Nur77 with a K_d of 0.29 μ M. We further demonstrated that celastrol binding to Nur77 induced Nur77 translocation to mitochondria, thus identifying a new ligand capable of inducing Nur77 mitochondrial targeting by binding. Celastrol is a potent anti-inflammatory triterpenoid quinone-methide isolated from the root of *Tripterygium wilfordii* commonly known as “Thunder

God Vine,” which has been used traditionally for the treatment of rheumatoid arthritis, lupus, and other auto-immune diseases.⁶ Celastrol has recently drawn considerable interest, after the discovery that the compound possesses potent anti-obesity activity.⁷ By using Nur77 null cells and Nur77 knockout mice, we demonstrated that Nur77 was required for the anti-inflammatory effects of celastrol *in vitro* and in 2 inflammatory animal models, including the lipopolysaccharide/D-galactosamine-induced acute hepatic inflammatory mouse model and the high-fat diet-induced obesity mouse model. These results identified Nur77 as a direct intracellular target of celastrol, providing important insight into the mechanism of celastrol actions. Celastrol binding resulted in not only the suppression of inflammation but also the induction of autophagy. The concurrent effects of celastrol on inhibiting inflammation and inducing autophagy suggested that both events might depend on each other. Our finding that inhibition of celastrol-induced autophagy impaired its anti-inflammatory effect revealed a role of Nur77 in mediating the crosstalk between inflammation and autophagy.

The anti-obesity activity of celastrol has been attributed to its leptin sensitizing effect.⁷ As hypothalamic inflammation is implicated in the development of leptin resistance, Nur77 that is highly expressed in the hypothalamus may likely confer the leptin sensitizing effect of celastrol by regulating inflammation and autophagy in the hypothalamus. A recent study showed that celastrol elicits anti-obesity effect through heat shock factor1 and peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α).⁸ Interestingly, PGC-1 α could also translocate to mitochondria and interact with nuclear receptor related 1 protein (Nurr1), a Nur77 subfamily member. Whether PGC-1 α acts to mediate the anti-obesity effect of celastrol through its interaction with mitochondrial Nur77 remains to be determined.

Tumor necrosis factor receptor-associated factor 2 (TRAF2),⁹ an adaptor molecule capable of assembling active nuclear factor of kappa B (NF- κ B) signaling modules, has recently emerged as an important regulator of mitochondrial signaling. TRAF2 can be recruited to mitochondria as part of death-inducing signaling complex upon TNF α stimulation, or as mitochondrial antiviral signaling protein aggregates to activate innate immune responses during viral infection. Our results showed that a prolonged treatment of cancer cells with TNF α resulted in altered mitochondrial networks and decrease in mitochondrial membrane potential ($\Delta\Psi_m$), suggesting a deleterious effect of inflammation on mitochondrial integrity. Interestingly, the effect of the prolonged TNF α treatment was accompanied with TRAF2 accumulation at mitochondria. Our data demonstrated that Nur77 and TRAF2 could strongly interact with each other and colocalize at mitochondria undergoing autophagy in cells treated with celastrol. These results revealed the ability of Nur77 to selectively induce autophagy of damaged mitochondria through its interaction with TRAF2.

The mechanism by which damaged mitochondria are recognized and engulfed by autophagosomes involves ubiquitination of mitochondrial proteins followed by interaction with the adaptor proteins connecting ubiquitin with microtubule-associated protein 1 light chain 3 (LC3).¹⁰ TRAF2 is an E3 ligase, which can promote Lys63-linked ubiquitination of its target proteins. Our results showed that TRAF2 interaction with Nur77 resulted in Lys63-linked Nur77 polyubiquitination, and importantly the ubiquitinated Nur77 resided at mitochondria. Thus, the TRAF2-dependent ubiquitination of Nur77 serves to prime damaged mitochondria for autophagy.

To provide molecular insight into Nur77-mediated mitophagy, we studied the interaction of Nur77 with p62/SQSTM1, a ubiquitin- and LC3-binding protein known to facilitate recruitment of damaged mitochondria to autophagosomes. Although the mitophagic role of p62/SQSTM1 has been subjected to dispute, we found that celastrol could induce a strong and specific interaction between Nur77 and p62/SQSTM1, revealing a role of p62/SQSTM1 in celastrol-induced mitophagy. Whether

other autophagy receptors play a role in the Nur77 mitophagic pathway and whether the Nur77 pathway interacts with the phosphatase and tensin homolog deleted on chromosome 10-induced kinase 1 (PINK1)/Parkin mitophagic pathway remain to be explored.

In summary, our results identify an important intracellular target mediating the anti-inflammatory effect of celastrol and unravel a new mitophagy pathway, in which celastrol-induced Nur77 mitochondrial targeting and interaction with TRAF2 and p62/SQSTM1 lead to clearance of damaged mitochondria, thus alleviating inflammation (Figure 1). They provide a new strategy to develop Nur77-based mitochondrial therapeutics and identify celastrol as a promising lead for this class of therapeutics.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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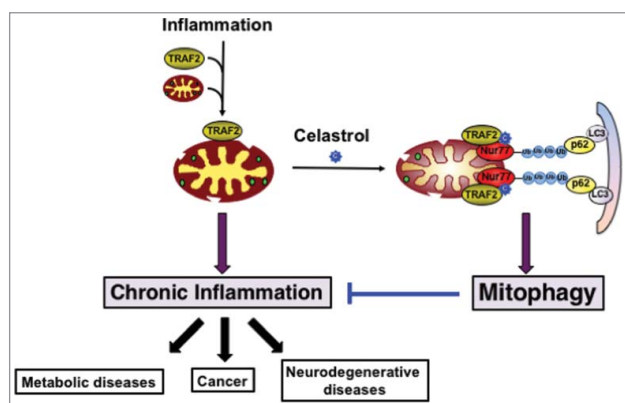


Figure 1. Mechanism of Nur77-mediated mitophagy. Our results revealed that damaged mitochondria resulted from inflammation insult could lead to chronic inflammation associated with the development of cancer and other inflammatory diseases. Upon binding to celastrol, Nur77 interacts with tumor necrosis factor receptor-associated factor 2 (TRAF2) at mitochondria, priming damaged mitochondria for clearance via autophagy through interaction of ubiquitinated Nur77, and autophagic protein p62/SQSTM1 (p62). Ub, ubiquitin; LC3, microtubule-associated protein 1 light chain 3.