Targeting Drp1 and mitochondrial fission for therapeutic immune modulation

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Graphical abstract
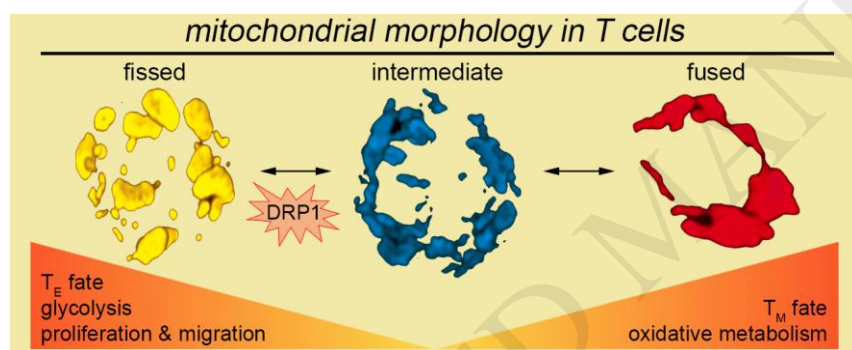
Abstract

Mitochondria are dynamic organelles whose processes of fusion and fission are tightly regulated by specialized proteins, known as *mitochondria-shaping proteins*. Among them, Drp1 is the main pro-fission protein and its activity is tightly regulated to ensure a strict control over mitochondria shape according to the cell needs. In the recent years, mitochondrial dynamics emerged as a new player in the regulation of fundamental processes during T cell life. Indeed, the morphology of mitochondria directly regulates T cell differentiation, this by affecting the engagment of alternative metabolic routes upon activation. Further, Drp1-dependent mitochondrial fission sustains both T cell clonal expansion and T cell migration and invasivness. By this review, we aim at discussing the most recent findings about the roles played by the Drp1-dependent mitochondrial fission in T cells, and at highlighting how its pharmacological modulation could open the way to future therapeutic approaches to modulate T cell response.

Keywords: Drp1, mitochondrial dynamics, T cells, tumor immune-surveillance, pharmacological approaches

Introduction

Mitochondria are eukaryotic organelles ensheated by a double-membrane, the inner one (IMM) characterized by several invaginations called *cristae*, where the supercomplexes of the electron transport chain (ETC) are located. The network of mitochondria is highly dynamic and the continuos processes of fusion and fragmentation (a.k.a. fission) are tightly regulated by several *mitochondria-shaping proteins*. Fusion is mainly regulated by Mitofusin-1 and Mitofusin-2 (which coordinate the fusion of the outer mitochondrial membrane, OMM) and Opa-1 (Optic atrophy-1, which regulates IMM fusion) [1,2]. Opa-1 also controls the organization of mitochondrial *cristae* and, therefore, directly affect the efficiency of the mitochondrial respiration (OXPHOS) [3]. The organelle fragmentation is regulated mainly by the activity of the dynamin-like GTPase Drp1 (Dynamin-Related Protein 1) [4]. In the inactive state, this protein normally localizes in the cytosol and is phosphorylated on serine637 (inhibitory phosphorylation) [5]. Its activation requires both the dephosphorylation of serine637, this directing Drp1 towards the OMM, and the phosphorylation on serine616, which activates Drp1 to execute mitochondria fragmentation [6]. Thus, Drp1 activity can be modulated by several protein kinases and phosphatases as well as other post-translational modifications, such as SUMOylation [5–7]. Docking of Drp1 to the OMM is regulated by different receptors and modulators, such as Mff [8] and hFis1 [9,10,11]. Further, Dynamin2 (Dyn2) has been recently proposed to work in concert with Drp1 to orchestrate the different steps required for mitochondrial fragmentation [12]. Besides regulating mitochondrial fragmentation, Drp1 can also directly control the morphology of the mitochondria *cristae* in a Opa-1-independent way, although the exact mechanism is not known yet [13]. Besides the general concept that mitochondrial dynamics are crucial for responding to distinct and specific cellular and physiological needs, mitochondrial fragmentation is very important for cell proliferation [14,15], death [16], migration [17], metabolism [16], ROS production [18] and mitophagic mitochondrial clearance [19], among other processes. Interestingly, all these processes are essential for a correct function of the adaptive immune system. Indeed, T cells continuosly migrate [20], get activated and expand extensively upon antigen encounter [21,22], dramatically change their metabolism [23] and eventually undergo a programmed form of cell death [24]. Consequently, several works in the recent years have linked mitochondrial fragmentation to the adaptive immune system activity. By this review, we aim at providing an updated and complete view of the different roles played by Drp1-dependent mitochondrial fission in the adaptive immune cells with a critical emphasis at the possibility to develop therapeutic strategies through the modulation of Drp1 activity in pathological contexts.

We will focus our attention only on T cells, since, compared to the large amount of data available about them, relatively few hints have been gained about the role played by mitochondria fission in other adaptive immune cell subsets, such as B and NK cells [25–29].

Mitochondrial fission in T cell activation and differentiation

One of the first established roles for mitochondrial dynamics in T cell physiology was the regulation of calcium currents following T Cell Receptor (TCR) stimulation. Indeed, mitochondria can uptake calcium ions from the cytosol, so preventing accumulation of excessive cytosolic calcium [30]. In the T cell activation context, where calcium acts as an important second messanger, mitochondria translocate beneath the plasma membrane of the Immunological Synapse (IS) at the contact sites with the antigen presenting cell (APC). There, the local mitochondria uptake of calcium prevents calcium-dependent inactivation of CRAC (Calcium Release-Activated Calcium) channels, sustaining a prolonged low-level calcium influx in the cytosol, required for optimal T cell activation [31–34]. In turn, calcium regulates both *i)* AMPK (AMP-activated protein Kinase) [35] and mTOR (mammalian Target of Rapamycin) [36] pathways, whose balance controls the transition of activated T cell from a metabolic quiescence towards an active anabolic metabolism [37,38] and

ii) NF-AT (Nuclear Factor of Activated T-cells) function, which drives the upregulation of glucose transporters and regulates the production of different cytokines [39]. The same calcium influx can also promote Drp1 activation through calcium-dependent calcineurin-regulated dephosphorylation on serine637 [40]. Further, MAPK (Mitogen-Activated Protein Kinases) pathway activation leads to Drp1 phosphorylation on serine616, resulting in Drp1 transition from an inactive towards a fully active state [41,42]. Then Drp1-dependent mitochondria fragmentation promotes mitochondria recruitment towards the IS by allowing the small and fissed mitochondria to be moved along microtubules, [42,43]. Surprisingly, Drp1 ablation from activated T cells does not lead to a blunt decrease in cytosolic calcium - as observed when mitochondria recruitment to the IS is prevented by other ways [44] - but to an increased and prolonged persistence of calcium ions in the cytosol [43]. Mechanicistically, Drp1 seems to be mainly required to finely adjust the mitochondria positioning around the actin-rich ring of the IS and its removal or inhibition in this context leads to an altered clusering of TCR/CD3 molecules at the IS, causing excessive calcium influx and abnormal transduction of the signalling from the TCR [43]. Such a prolonged calcium influx upon activation in Drp1-deficient T cells leads *i)* to an increased expression of the cytokine IL-2 [43], presumably due to NF-AT activation and *ii)* to an altered AMPK-mTOR balance, this, in turn, leading to a defective mTOR-dependent upregulation of cMyc and, consequently, a poor engagment of glycolysis [42] (Figure 1). Besides that, Drp1-dependent mitochondrial fission also regulates the generation of reactive oxygen species (ROS) [45], which are essential for several mediators of T cell activation, such as NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and AP-1 (Activator Protein 1) [46] (Figure 1). Although the exact mechanism of action is not known, it has been shown that ROS release from mitochondria depends on an altered functionality of the electron transport chain (ETC) complexes and of the other proteins involved in the mitochondrial oxidative respiration (OXPHOS) [47]. It can be speculated that, similar to what reported in other cell types [13], the Drp1-dependent [42] fragmentation of the mitochondrial network in activated T cells also favours the disassembly of the mitochondrial *cristae* [41], which are invaginations of the IMM whose morphology controls the functionality of the ETC supercomplexes here located, and consequently ROS production [48]. Last, Drp1-dependent mitochondrial fragmentation is required to allow optimal clonal expansion of activated T cells, both *in vitro* and *in vivo* [42]. Drp1 may control also cell cycle progression in different ways [14,15], and indeed, it seems that Drp1 deficient T cells have a prolonged mitosis due to an altered centrosome structure, caused by a hyperfused mitochondria network surrounding these structures [42].

Once released from thymus, mature naïve T cells display a low metabolic activity, characterized by a poor engagment of mTOR signalling, a very low mitochondrial mass, due to mitophagy-mediated degradation, and low levels of ROS production [49]. The activation of a naïve T cell, following TCR stimulation, is accompained by a drastic alteration in its metabolic status, which is shifted toward the engagement of anabolic reactions, essential to sustain T cell growth and proliferation [50]. Upon activation, glucose uptake, glycolyis and glutaminolysis are strongly upregulated [50] through the engagement of specific signalling pathways [51–54]. mTOR also promotes the uptake and the synthesis of fatty acids for membrane biosynthesis [55]. Once activated, T cells can differentiate toward either an effector or a memory fate. Effector T cells mount the rapid immune response against foreign antigens, but are committed to die shortly. On the contrary, memory T cells survive the resolution of the immune response and become long-lived cells with the potential to generate new effector cells during a secondary response. Interestingly, several works have demonstrated that while effector T cells show mainly a glycolytic metabolism [56], memory T cells (and partly also regulatory T cells) mostly rely on mitochondrial OXPHOS, sustained, at least in part, by fatty acid β-oxidation (FAO) and AMPK activity [57–60], and on the pentose phosphate pathway, this to generate high amounts of NADPH and glutathione levels for red-ox balance [61]. Further, the metabolic status of an activated T cell can influence its differentiation toward an effector or a memory fate [62]. For examples, forcing glycolysis reduces the number of memory T cells generated upon activation, while its inhibition [56], or a

pharmacological modulation of mTOR signalling [63], promote a memory fate similar to the the forced utilization of fatty acids as energy source [59]. Although the exact mechanism of such regulation is still not completey understood, what is currently emerging is the idea that an activated T cell can adjust its metabolism to control the amount and the availability of different metabolic intermediates. The latter are then used for epigenetic reactions controlling the transcription of key genes that instruct the cell to engage an effector or a memory differentiation programme [64]. Indeed, most metabolites produced in mitochondria-associated reactions are required for the (de)acetylation and (de)methylation of both DNA and histones [65] and the modulation of their levels affects T cell differentiation through epigenetic reprogramming [66–69]. The morphology of the mitochondria network is tightly linked to the metabolic status of the cell. Indeed, fragmented mitochondria are frequently observed in glycolytic cells, while a more elongated network, also characterized by more efficiently assembled *cristae*, is found in cells with an OXPHOS-based metabolism [16]. T cells are not an exception to this rule. Glycolysis-based T cells undergoing towards an effector differentiation also show highly fragmented mitochondria [41], thanks to Drp1-mediated mitochondrial fission [42]. Further, activated T cells generate new mitochondria, which are specifically dedicated to the one-carbon metabolism for nucleotide biosynthesis [70]. On the contrary, memory T cells show an increased mitochondrial mass, a more elongated network [71] and a higher efficiency of the ETC supercomplexes, due to Opa-1-dependent *cristae* regulation [41]. Such increased mitochondrial fitness and mass allows memory T cell to proliferate quickly and to generate more cytokines upon re-activation compared to naïve T cells, this being one of the mechanism responsible for the stronger memory-associated response upon secondary encounter of T cells with an antigen [71,72]. It has been shown also that even before the first cell division, following TCR stimulation, a transient phase of mitochondria elongation, and engagment of FAO, is required to prime future memory T cells with the ability to quickly respond to a second stimulation [73]. Consistent with what previously discussed, modulating the activity of several mitochondria-shaping proteins, during or immediately after T cell activation, influences not only the morphology of the mitochondrial nework and the cellular metabolism, but also the differentiation toward an effector or a memory fate [41,42,74]. Interestingly, Drp1 seems to play a double role in this regulation. Indeed, on one side, the Drp1-dependent [42] mitochondrial fragmentation in effector T cells favours a less efficient organization of *cristae* structure, reducing OXPHOS rate [41]; on the other side, Drp1-dependent regulation of calcium influx upon TCR stimulation sustains the activation of the mTOR/cMyc axis (see previous paragraph), leading to the transcriptional upregulation of the genes encoding glycolytic enzymes [42] (Figure 1). Therefore Drp1 is essential to fully instruct T cell into an effector fate. It will be interesting in the future to investigate how Drp1 activity modulates the metabolomics of activated T cells and how this modulation influences the epigenetic regulation of the genes implicated in T cell differentiation.

In sum, Drp1-dependent mitochondria fragmentation plays an important role during T cell activation following TCR stimulation, also by impacting on T cell metabolism and differentiation. From a clinical point of view, these findings suggest that the modulation of Drp1 activity could be used in the future to control the immune response following T cell activation.

Mitochondrial fission in T cell migration and death

All main processes defining a T cell life, such as APC-triggered activation, recognition of target cells for their killing and immune-surveillance of self-tissues, require this cell being able to correctly migrate in the right way and toward the right location. Therefore cell migration, which relies on a fine regulation of actin filaments organization and myosin force, is critical for an efficient T cell functionality. At variance with cancer cells and their ameboid-like migration pattern, T cells show a different organization of their actin-myosin cytoskeleton [75]. Indeed, during their fast directional migration, T cells accumulate their actin-myosin motor at the uropod, i.e. their rear

edge, where the contractile force generated by myosin drives cell migration, pushing the cell from the back. Interestingly, the high amount of ATP required for the myosin motor is produced by mitochondria, which are actively recruited at the uropode [76]. Upon chemokine stimulation, the activation of ERK pathway [77] leads to Drp1 phosphorylation on serine616 and the consequent mitochondrial fragmentation, which redistributes the mitochondria at the uropode along microtubules [42] (Figure 1). Similarly, during their trans-migration across an endothelial layer, leukocytes squeeze their nuclei and insert them into a subendothelial pseudopodium [78]. This process heavily relies on the activity of the myosin motor [79] and requires Drp1-dependent mitochondria fragmentation, too [42]. Consistently, *in vivo* ablation of Drp1 from T cells disrupts their normal extravasation from blood toward secondary lymphoid organs, and also a "danger site" such as a tumor mass [42]. Interestingly, Drp1 seems to regulate also the migration of developing thymocytes, a very important process for the correct maturation of a T cell repertoire. Indeed, Drp1 ablation reduces thymocyte migration rate, and this indirectly affects their survival during the positive selection [42]. Beside a fast directional migration pattern, T cells may also display a slow-moving motility when probing the extracellular environment and searching for adjacent cells to communicate with. In this case, portions of the mitochondria network are accumulated toward the leading edge of a migrating T cell, where they are required again to supply ATP to sustain a feed-forward feedback loop between chemokine and purinergic receptors, which further promotes cell migration [80]. It is not known whether mitochondria need to be fragmented in this case and if Drp1 may play a role during slow-rate T cell migration too. However, also during *in vitro* T cell degranulation, portions of the mitochondria network relocalize at the level of the IS and Drp1 is dispensable for this process [42]. This suggests that Drp1-mediated mitochondrial fragmentation may be mainly required when the whole network need to be relocated (such as during fast-directional or transendothelial migration), or when the position of mitochondria need to be finely asdjusted (such as during IS formation for full T cell activation), and not for processes requiring only portions of the network to be recruited toward specific subcellular compartments.

Once the source of inflamation and damage has been removed, the shut-down of the immune response, including that of activated T cells, is essential to avoid the generation of hyper-reactive cells and auto-immunity. While a small fraction of activated T cells return into a quiescent state, ready for the next identical insult (i.e. they become memory cells), most of them undergo a process of finely controlled cell death which is coupled to T cell activation. Indeed, the encounter with a target cell triggers the stimulation of the T lymphocyte, which at the same time exposes on its surface death ligands (such as FasL) and degranulates (releasing killing molecules) to promot target cell death. The same cell also undergoes cell death by itself, in a process known as Activation-Induced Cell Death (AICD). It has been initially believed that AICD was mainly due to the cross-activation (i.e. in trans) of death ligands and receptors couples (such as Fas and FasL) on stimulated T cells, which than execute the extrinsic pathway of apoptosis, including Bid truncation and caspase-8 activation. However, several recent findings indicate that caspase-independent routes of this specific T cell death also occur [81]. In most cases mitochondria have been shown to represent a hub for integrating different pro-survival or anti-survival signals leading or not to cell death by eventually releasing several death molecules, such as cytochrome-C, AIF (Apoptosis-Inducing Factor) and Smac/Diablo (this being well known as the intrinsic apoptotic pathway). This holds true not only for cancer cells [16], but for T cell as well [82]. In cancer cells, Drp1 has been implicated as a regulator of the intrinsic pathway of apoptosis, which requires two different steps: the opening of the IMM *cristae* (leading to cytochrome-C release toward the IMS) and the formation of the MOMP (Mitochondria Outer Membrane Pore, which allow cytochrome-C release from the IMS toward the cytosol). Indeed Drp1-dependent hemi-fission of the OMM favours the insertion of the pro-apoptotic Bcl-2 family members Bax and Bak, a required step for the formation of the MOMP [83]. In addition, Drp1 may also directly control the *cristae* opening upon apoptosis induction [13]. In T cells, it has been recently shown that AICD progression is characterized by the translocation of Drp1 from the cytosol toward mitochondria [84]. Consistently, hFis1 (one of the docking receptors

for Drp1) is localized inside OMM lipid microdomains, which are also the sites of Bax recruitment [85]. Subsequently, mitochondria fragment, depolarize and open their *cristae* and, since autophagy is preventively inhibited, not-removed damaged mitochondria can release the cell death executioners [84]. Therefore, Drp1 play an important pro-apoptitic role also during AICD (Figure 1). However, it has been reported that one of the mechanisms by which mesenchymal stem cells may promote chemoresistence in T-cell acute lymphoblastic leukemia (T-ALL) cells is through the upregulation of the ERK signalling, and the consequent activation of Drp1 through phosphorylation on serine 616 [86]. Thus, similarly to what observed in some solid cancer cell models [16], Drp1 activity may become also protective against some apoptotic stimuli in transformed T cells. Contrary to what observed in activated mature T cells, Drp1 seems to be dispensable for the AICD of developing thymocytes upon TCR stimulation during the processes of negative and positive selection in the thymus [42]. Since developing thymocytes are extremely prone to cell death following TCR stimulation to eliminate auto-reactive cells, it may be possible that multiple and redundant pathways, compared to mature T cells, operate in thymocytes to induce the apoptotic cascade following TCR engagment and Drp1-dependent mitochondrial fission may become dispensable in this situation.

In sum, Drp1-dependent mitochondria fragmentation plays an important role in promoting both T cell migration and T cell death, and the therapeutic modulation of its activity could be exploited as a tool to regulate the response of T lymphocytes impacting, by this way, on their invasivness and/or survival.

Mitochondrial fission in exhaustion

While short-living effector T cells undergo a programmed cell death following recognition (and killing) of a target cell, memory precursors are long-living T cells and guarantee immunological memory. However, when the source of the infection, or damage, is not resolved (such as during a chronic infection or tumor growth), the continuous stimulation of memory precursor cells diverts their differentiation programm toward the generation of an non-functional state with low proliferation potential and poor cytokine production, known as "exhaustion" [87]. In the most recent years, impressive efforts have been engaged to revert the exhaustion state of antigen-reactive T cells, trying to restore their functionality to bolster the immune response against both infections and tumors [88,89]. Consistent with the emerging roles that mitochondria play during various aspects of a T cell response, several works are now focused on these organelles, in this context. It has already been highlighted how mitochondria-related metabolism is affected in exhausted T cells, thus suggesting new approaches to be exploited with the aim to renvigorate the immune response. However, besides the metabolic aspect, if and how mitochondrial dynamics are altered upon exhaustion is still not known. Among the very few data available, it has been reported that tumor-infiltrating T cells (including also non-exhausted T cells) in clear cell renal cell carcinoma (ccRCC) display an abnormal fragmented mitochondrial morphology associated with increased ROS production [90]. The latter is also observed in exhausted T cells in chronic hepatitis B patients, characterized by reduced transcription of Opa-1, one of the main pro-fusion proteins [91]. Last, it has been reported very recently that the metabolic quiescence of naïve CD4^{+} T cells is actively maintained by the activation of LAG-3 (Lymphocyte Activation Gene-3) signalling, one of the main inhibitory co-receptors driving T cell exhaustion. Indeed, LAG-3 signalling reduces mitochondrial metabolism and spare respiratory capacity by dampening the IL-7-mediated STAT5 (Signal transducer and activator of transcription 5) activation [92].

One of the best characterized mediator of T cell exhaustion is PD-1 (Programmed-cell Death Protein-1), an inhibitory co-receptor expressed in T cells upon activation [93]. PD-1 signalling inhibits glycolysis in exhausted T cells, and promotes a shift toward FAO utilization [94]. However,

PD-1 also down-regulates the expression of PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha), the master gene driving mitochondria biogenesis. This causes a loss of mitochondrial mass [95] and accumulation of damaged and depolarized mitochondria, which cannot cope with their increased metabolic load, so leading to the production of high ROS amounts [96]. In turn, this can make T cells more sensistive to cell death [97], which can be indeed prevented by treating exhausted T cells with antioxidants [91]. Unfortunately, it is still not known how mitochondria morphology is modulated in this scenario, but it has been reported that PD-1 modulates both mTOR and ERK (Extracellular-signal Regulated Kinase) pathways [98,99] and, as said, both of them are regulators of the Drp1-dependent mitochondrial fission [42,100]. Interestingly, this suggests that an impairment of Drp1 activity in exhausted T cells may inhibit the removal of such damaged and depolarized mitochondria through mitophagy, thus exacerbating the dysfunctional state of these cells. Further, PD-1-driven down-regulation of ERK signalling causes a paralisis of exhausted T cells [99]. Given that the ERK/Drp1 axis is an important regulator of T cell migration [42], it will be interesting in the future to investigate if the impaired motility of exhausted T cells may be due, at least partially, to Drp1 down-regulation (and if it could be reverted by modulating Drp1 activity). The same holds true regarding the programmed cell death of tumor infiltrating T lymphocytes, another process known to be regulated by the ERK signalling [101].

In sum, when exhausted, T cells show alterations in different aspects of their behaviour, aspects that have been shown being dependent on a fine regulation of mitochondrial morphology, such as metabolic adaptability, cell migration, proliferation and response to cell death. Investigating how the mitochondria morphology is consequently affected, and which roles Drp1 may play in all these processes, could open the way in the future to new therapeutic strategies to modulate T cell function or exhaustion by impacting on Drp1 activity. For example, this would be very important for exhausted T cell in the tumor-microenvironment, for which it has been recently demonstrated that improving mitochondrial fitness, in a 4-1BB-dependent way, can bolster anti-tumor T cell response [102].

Drugs targeting mitochondrial fission and their application in the immune system

Given the multiple roles played by Drp1-dependent mitochondria fission in T cells, the possibility to manipulate, pharmacologically or genetically, its activity could open the way to new therapeutic opportunities. For example, inhibiting *in vivo* Drp1-dependent mitochondria fragmentation could reduce both T cell invasivness and expansion, thus dampening T cell response. This could have an obvious application in auto-immune diseases or in those cases in which the aggressiveness of T cells must be kept under control. On the other side, Drp1-dependent mitochondria fragmentation could be artificially promoted when T cell infiltration must be increased, such as inside a tumor microenvironment [89]. However, such scenario becomes more complicated when we consider also the effect on T cell differentiation, i.e. the generation of an effector or a memory T cell. In this case, Drp1-dependent mitochondria fragmentation mainly sustains the generation of glycolytic-dependent effector T cells, which are also short-living, and prevents the generation of long-lasting OXPHOS-based memory cells. Therefore, forcing Drp1-dependent mitochondria fragmentation can generate a large number of T cells with high invasive potential, but they will have also a limited lifespan. On the contrary, Drp1 inhibition promotes the generation of long-lasting memory cells, but they will be fewer and with a reduced migratory capacity. This double-edge effect of Drp1 inhibition (or activation) may pose some limitations to its application in therapy. For example, a prolonged lifespan (favored by Drp1 inhibition) and a high invasive potential (favored by Drp1 activation) should be the best combination to be found in T cells fighting a growing tumor. These considerations urge us to carefully plan the correct *timing* of treatment, if we aim at modulating Drp1 activity during future therapeutic approaches. In addition, given the house-keeping role of Drp1-dependent mitochondria fragmentation, *in vivo* treatments

with Drp1 (ant)agonist should also consider the possible side-effects on other cell types. This suggests that *ex vivo* treatment of T cells with Drp1 (ant)agonists during adoptive cell immuno-therapy protocols could be preferable, for example to promote the generation of long-lived memory cells [41], without any side effect on invasive ability once these cells will be injected back into the patients. In any case, the pharmacological modulation of Drp1 activity, either to increase or to decrease it, requires an analysis of the drugs currently available to modulate its functionality, as it follows.

The machinery driving fission of mitochondria is therefore target of pharmacological approaches to modulate the process. In the literature a wealth of approaches is reported to genetically manipulate Drp1 level by repressing its expression (sh/siRNA protocols) or block its activity via dominant negative mutant [103,104]. These nonetheless bear limitations entailed in the kinetic of action and suitability for therapy, which favours the development of chemically based approaches. This has propelled the quest to devise tools such as peptides and/or small molecules to impact the dynamics of mitochondrial remodelling by targeting Drp1 and its GTPase activity [104].

-) Peptides were designed to limit the Protein-protein interaction (PPI) between Drp1 and its mitochondrial adaptor (i.e. Fis1) which yielded to the isolation of short types of peptides working as effective inhibitors. Selectivity of action was recently secured with the peptide inhibitor P110 which prevents mitochondrial fission inhibiting activation of Drp1 by blocking its interaction with Fis1 [105]. This approach proved particularly beneficial under cell stress conditions and in neurodegenerative models of analysis, together with a proven *in vivo* safety profile [105]. Interestingly P110 has no effect on mitochondrial morphology and function under physiological conditions. The knowledge gained on other Outer mitochondrial membrane (OMM) adaptors via which Drp1 is anchored on the network, suggested to the targeting of the mitochondrial fission factor (Mff) as alternative strategy [104]. Mff deletion establishes more elongated mitochondria and a specific peptide against this target was therefore devised to prevent the interaction between Mff and Drp1. The short-peptide P259 efficiently blocks the Drp1-Mff bond [106], without affecting any of the other anchoring elements. This approach proved successful in reducing the degree of mitochondrial fission but presented limitations due to essential role played by Mff in maintaining mitochondrial homeostasis by trimming the network. *In vitro* and *in vivo* treatments with P259 did cause reduction of brain ATP levels along with motor and neurological deficits [106]. This implied that inhibiting basal mitochondrial fission via this pathway could be detrimental and not protective - as originally hoped- and on the contrary favouring neuropathology. Maybe this be for detriment of neurons as well as deficient function by microglia.

-) In spite of their value in assessing and/or probing biological processes, peptides have limited success in being translated into commercial drugs. The very opposite is true for small molecules. And these have been therefore relentlessly pursued in the attempt to devise pharmacological regulation of Drp1 for translation into therapy. The most renown example is the cell permeable quinazolinone mdivi-1 originally described by Cassidy-Stone in 2008 [107] and enrolled since then in over 100 primary research publications. The compound was discovered through a library screening in yeast in which the mitochondrial morphology and organism survival were set as hits. Biochemical analysis revealed that mdivi-1 tackles GTPase activity of Drp1. The function reported in yeast models was subsequently confirmed in mammalian cells in which the precise activity was not immediately determined. We now know that midivi-1 operates by preventing Drp1 self-assembly into rings and its association with mitochondria [108]. Mdivi-1 is therefore operating as an allosteric modulator of Drp1 to prevent the essential oligomerization required for the GTPase activity. Nevertheless, the ability of mdivi-1 to inhibit Drp1 and impact mitochondrial fission has been recently challenged by Bordt et al. [109], in which authors did not find any effects of mdivi-1 treatment on mitochondrial morphology in mammalian cells (primary neurons and COS-7 cells) whilst they did in yeast. A side toxic effect via the inhibition of the complex I of the electron transport chain was also reported spelling doubts on the potential use of this compound. In spite of

this, the suitability of mdivi-1 as a tool to manipulate Drp1 function, is well corroborated even though additional analyses (particularly in immune cells) are warranted to offset the activity of mdivi-1 towards Drp1 ring formation and GTPase activity, as well as its potential side effects on respiratory complexes. The evidences by Bordt was not the sole ones reporting on alterations in oxygen consumption in mdivi-1 treated cells which are independent from its Drp1 direct function [110,111].

-) Beyond short-peptides and small molecules, the hormone Irisin was recently noted for the marked ability to inhibit mitochondrial fission driven by Drp-1. Data were collected in liver, in which treatment with Irisin mediates a protective effect on ischemia/reperfusion damage, thus increasing mitochondrial content overall via an effect on biogenesis-related peroxisome proliferative activated receptor-γ (PPARγ) co-activator 1α (PGC-1α) as well as the mitochondrial transcription factor A (TFAM) expression [112]. Taming hormones in order to affect mitochondrial dynamics does appear rather challenging and likely unfit to match the spatio-temporal requirements to be therapeutically incisive in immunomodulation. Given the complexity of systems in which modifications of the balance between mitochondrial fusion and fission is critical for cellular homeostasis and even more so in specialized cells such as neurons, pharmacological tools are what is required to engage and, if needed, address mitochondrial fission.

Concluding remarks

In sum, mitochondria have emerged as key hub orchestrating several aspect of T cell biology. In these last years, it has become increasingly clear that also the morphology of the mitochondria network plays an important role in the regulation of multiple processes in T cells, such as activation, differentiation, apoptosis, proliferation and migration. From a pharmacological point of view, all these findings open the way to future applications in the clinical practice, by exploiting our ability to modulate Drp1 activity, with the aim at turning T cell behaviour at our will. However, given the multiple roles played by Drp1 in T cells, our primary challenge in the future will be to understand how to finely modulate its activity to maximize our results in the therapy, and to reduce at the same time any possible side effect. Indeed, the *in vivo* application of current available drugs targeting Drp1 is still limited by their numerous side effects. In this way, the possibility to manipulate *ex vivo* Drp1 activity (either using drugs or genetic manipulations) in isolated T cells seems more promising at the moment, also considering the increasing interest in chimeric antigen receptor (CAR) T cells. Further, we very recently demonstrated that optogenetics could be implemented in isolated T cells to modulate mitophagy [113], opening the way to future light-based Drp1 manipulations, which could more finely tune its activity in time and space, even *in vivo*.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Legend

Figure 1. *Schematic representation of the various roles played by Drp1 in T cells.* Upon TCR engagement, Drp1 converts from an inactive (pSer637) into an active state (pSer616) and mediates the fragmentation of the mitochondria, which then translocate toward the IS. Here, fragmented mitochondria finely tune calcium influx, regulating mTOR and AMPK balance and leading to optimal cMyc activation and the consequent transcription of glycolytic genes. In addition, fragmented mitochondria may lose membrane potential due to disassembled *cristae* structure, leading to increased ROS production, which are essential for the correct T cell activation. During AICD, such depolarized mitochondria may also lose cytochrome-C, which once in the cytosol activates the death-inducing caspases cascade. Further, during T cell migration, Drp1 is activated by signals from chemokine receptors and promotes fragmentation of the mitochondria, which can then be transported along microtubules toward the uropod (MTOC, microtubules organizing centre), where they produce high local amounts of ATP to fuel myosin motors.