INTRODUCTION

Dendritic cells (DCs), the most potent antigen-presenting cells in bridging innate and adaptive immunity, play a central role in orchestrating the decision between immunity and tolerance. The development, maturation, and function of DCs are under the intricate control of various cellular and molecular mechanisms. Signaling via the mechanistic target of rapamycin (mTOR), a key kinase-mediated pathway in integrating cellular and environmental cues, has emerged as a crucial regulator of various aspects of DC biology. These studies have benefited from pharmacological inhibitors of mTOR, especially rapamycin, and genetic dissection of mTOR components and regulators such as Pten, Tsc1 and Raptor. Here we review the recent advances in understanding the functional significance and mechanistic basis of mTOR signaling in DC development and function.

mTOR signaling

The mechanistic target of rapamycin (mTOR, a serine/threonine kinase) is the catalytic unit of two distinct multi-protein complexes: mTOR complex 1 (mTORC1) and mTORC2 that are respectively defined by the scaffold proteins Raptor and Rictor (Figure 1) [2]. mTORC1 activity is dynamically regulated by environmental and intracellular signals, including immune, growth factor, and metabolic cues. Many upstream signals activate mTORC1 pathway through the small GTPase Rheb (Ras homologue enriched in brain). The tuberous sclerosis 1 (Tsc1) and Tsc2 form a complex that inactivates Rheb, thereby suppressing mTORC1 activity. Further upstream, the phosphoinositide 3-kinase (PI3K)-AKT pathway inactivates Tsc1/Tsc2 complex while AMP-activated protein kinase (AMPK) enhances its activity. The activity of PI3K-AKT pathway is also tightly controlled by negative regulators, with the most notable factor being the lipid phosphatase Pten. Deletion of Pten leads to the constitutive activation of PI3K-AKT signaling and is a common event in malignant transformation. S6K1 and 4E-BP1 are two best-characterized downstream targets of mTORC1 that regulate protein translation [2]. mTORC1 pathway also promotes anabolic metabolism such as glycolysis and lipid biosynthesis while inhibiting autophagy [3].

PI3K also signals to mTORC2 in a process dependent upon the ribosome, although detailed mechanisms are not fully understood [4]. A signature activity of mTORC2 is the phosphorylation of AKT at Ser473, thereby contributing to full activation of AKT and the regulation of AKT downstream targets such as Foxo1 and Foxo3 [2].
Modulation of DC development, maturation and function by Rapamycin

The immunosuppressive property of rapamycin is traditionally ascribed to its potent activity to inhibit lymphocyte proliferation [5]. Recent studies have revealed that rapamycin impinges upon the differentiation, maturation and function of DCs, and these effects also contribute to its immunomodulatory property [6]. First, prolonged treatment with rapamycin in vivo decreases the numbers of DCs under steady state, as well as the expansion of DCs upon Flt3L treatment in vivo [7]. DC development can be recapitulated in BM culture supplemented with Flt3L or GM-CSF. Rapamycin strongly inhibits the generation of both pDCs and cDCs in Flt3L-supplemented culture, partly by blocking the proliferation of DC progenitors. In contrast, rapamycin does not prevent DC development in GM-CSF-supplemented BM culture [8].

Second, rapamycin impedes the maturation of DCs and instead, enhances their tolerogenic potential. Rapamycin inhibits the maturation of BM-derived DCs (BMDCs) as indicated by the decreased expression of co-stimulatory (CD80 and CD86) and MHC-Class II (MHC-II) molecules, even after exposure to potent inflammatory stimuli such as TLR and CD40 ligation [7,9,10]. Rapamycin-treated DCs are poor stimulators of T cell responses, by impairing T cell proliferation and IL-2 and IFNγ production [7,9] and inducing T cell anergy and apoptosis [9,10]. Instead, rapamycin-treated DCs enhance the generation of regulatory T cells [10]. In vivo, rapamycin-conditioned DCs prolong organ allograft survival [9,10] and reduce graft-versus-host disease [11]. Mechanistically, rapamycin impairs the expression of functional IL-4R and thus the responsiveness of DCs to the maturation-promoting signal IL-4 [7]. Additionally, rapamycin conditioning of DCs elicits the de novo production of IL-1β, which promotes the expression of the transmembrane protein IL-1R-like 1 (IL-1RL1, also known as ST2), a potent negative regulator of TLR signaling [12]. Thus, rapamycin-treated DCs undergo impaired maturation and promote immune tolerance by diminishing effector T cell responses and promoting regulatory T cell generation.

Third, rapamycin exerts potent effects on DC functions and DC-mediated immune responses. An important mechanism is to shape the production of pro- and anti-inflammatory cytokines. However, as compared with the inhibitory effects of rapamycin on DC differentiation and maturation, the role of mTOR signaling in DC cytokine production is more complex. Weichhart et al reported that rapamycin promotes IL-12 but suppresses IL-10 production in DCs through regulation of transcription factors NF-kB and STAT3, respectively [13]. In an independent study, Ōhata also described the reciprocal effect of rapamycin on IL-12 and IL-10 expression in LPS-stimulated DCs. In this case, inhibition of IL-10 function reverses the enhancing effect of rapamycin on IL-12, indicating that rapamycin inhibits IL-12 expression indirectly via the autocrine action of IL-10 [14]. Further, the enhanced IL-12 production in DCs generated after chronic exposure to rapamycin has been ascribed to the failure to down-regulate GSK-3 activity [15]. Thus, rapamycin promotes IL-12 expression in DCs via distinct mechanisms in a context-dependent manner. Despite these observed positive effects on IL-12 production, rapamycin is also capable of suppressing IL-12 expression in human monocyte-derived DCs in response to LPS or CD40L stimulation [16], thereby highlighting the complex roles of mTOR in the regulation of IL-12 expression. Another surprising finding is that rapamycin promotes IL-1β secretion via caspase-1 activation [12,17]. As for other pro-inflammatory cytokines such as IL-6 and TNFα, mTOR inhibition has more variable and context-dependent effects. For instance, rapamycin has been shown to inhibit [17] or promote the expression of these cytokines [13], or have no strong effects [14].

Another important mechanism whereby mTOR signaling regulates DC functions is its requirement for the production of type I interferon (IFNα/β), especially in pDCs. In pDCs stimulated with TLR9, rapamycin treatment reduces the production of IFNα/β through disruption of the TLR9-MyD88 complex and subsequent impairment of phosphorylation and nuclear translocation of IFN regulatory factor 7 (IRF7). In vivo rapamycin treatment results in decreased IFNα/β in serum and in pDCs in response to stimulation with a TLR9 ligand or viral vaccine, leading to impaired immune responses [18]. A similar effect of rapamycin to inhibit TLR9-induced production of type I interferon has been observed in Flt3L-derived murine DCs and human PBMCs [17]. Consistent with these observations, inhibition of PI3K, especially PI3Kδ, impairs the ability of human pDCs to produce type I interferon but not other pro-inflammatory cytokines such as TNFα or IL-6 [19]. Aside from affecting cytokine and interferon production, rapamycin also regulates other cellular events in DCs to impinge upon DC functions [6]. For example, rapamycin has been shown to impinge upon DC survival [7,20,21], migration [11,22], antigen uptake [23,24] and autophagy [25].

Overall, these results indicate that rapamycin exerts a
plethora of effects on DC development, maturation and functions. However, it is important to note that rapamycin is not an efficient inhibitor of mTORC1-mediated 4E-BP1 phosphorylation [26]. Additionally, rapamycin may inhibit mTORC2 activity with prolonged treatment and/or at a high dose [27, 28]. As a result, second-generation mTOR inhibitors have been developed that directly target the mTOR catalytic activity and thus have more specific and potent effects on mTOR [29]. Indeed, using these second-generation mTOR inhibitors, Rosborough et al recently reported that a rapamycin-insensitive mTORC1 signaling controls IL-10 and B7-homolog 1 (B7-H1) expression by DCs and the induction of regulatory T cells [30].

**Inhibition of Flt3L-dependent CD8+ cDC development by Pten**

Pten, an important signaling molecule in tumor suppression and immune regulation, is also critical for DC development and function (Figure 2). By using a tamoxifen-inducible Ptenflox/flox; Rosa26-Cre-ER mouse strain, Sathaliyawala et al showed that acute Pten deletion in hematopoietic progenitors promotes the development of all DC subsets in the culture with Flt3L [8]. In vivo analysis demonstrates that the chimeras reconstituted with Pten-deficient BM cells contain increased numbers of CD8+ cDCs, but not CD8- cDCs or pDCs, indicating that Pten deletion in hematopoietic cells preferentially facilitates the development CD8+ cDCs. The authors further used Pten-flox/flox; CD11c-Cre+ mice that lack Pten in the CD11c-expressing DCs to investigate the specific role of Pten in mature DCs. In these animals, the number of splenic CD8+ cDCs is increased by 5-6-fold, and the competitive chimera system shows that this expansion is a cell-intrinsic effect. Cell developmental origin analysis demonstrates that the expansion of CD8+ cDCs in the absence of Pten originates at the immature CD8low differentiation stage. As the functional and developmental equivalent of CD8+ cDCs, the CD103+ cDC subset in tissues is also increased in Pten-flox/flox; CD11c-Cre+ mice. After DC-specific Pten loss, the control of Listeria infection is impaired, although antigen-specific T cell responses are mounted normally. Thus, Pten expression in DCs controls DC subset homeostasis that contributes to protection against bacterial infections. Consistent with the preferential effect of Pten deletion on CD8+ cDCs, such DC subset contains higher mTORC1 activity (p-S6) than CD8- cDCs under steady state and upon Flt3L treatment in vivo. Importantly, rapamycin treatment reverses the expansion of Pten-deficient DCs in vitro and in vivo. These results demonstrate that restriction of Flt3L-induced mTOR signaling by Pten ensures optimal DC pool size and subset composition [8].

Other studies using pharmacological inhibition or silencing of Pten also indicate a role of Pten in DC differentiation and function. Enhanced mTOR signaling via pharmacological inhibition of Pten or hyper-activation of AKT results in increased proportion and absolute number of human CD34+ cell-derived pDCs [31]. A small interfering RNA (siRNA) targeting Pten in DCs promotes their survival and maturation, including increased expressions of co-stimulatory molecules, which in turn facilitates antigen-specific CD8+ T cell activation in vitro and in vivo [32].

**Tsc1-mediated regulation of DC development and function**

The tumor suppressor Tsc1 integrates upstream signals to suppress mTORC1 activity, and is also involved in modulating mTORC2 activity [2]. Our recent study demonstrates that Tsc1 is an important regulator of DC development [33]. We generated the Tsc1-flox/flox; Rosa26-Cre-ER genetic model and found that the ablation of Tsc1 after in vivo tamoxifen treatment impairs DC development. Loss of Tsc1 also inhibits the development of Flt3L-derived BMDCs in vitro. These defects are associated with diminished cell survival and proliferation. Moreover, Tsc1 deficiency causes DC spontaneous maturation, as indicated by the increased expression of co-stimulatory molecules, but a propensity to differentiate into other lineages. Moreover, Tsc1-deficient DCs produce decreased IL-12 and are impaired to mediate effector TH1 responses.

Mechanistically, Tsc1-deficient DCs exhibit increased activities of multiple metabolic pathways including glycolysis, mitochondrial respiration and lipid synthesis, as well as elevated expression of the transcription factor Myc, an established regulator of cell metabolism. Importantly, deletion of Myc in Tsc1-deficient cells partially blocks defective cell metabolism and DC survival and maturation, highlighting a key role of the Tsc1-Myc axis in metabolic programming of DC differentiation. Further, Tsc1 deficiency results in increased mTORC1 but decreased mTORC2 activity. Either rapamycin treatment or deletion of Rheb reverses the defective development of Tsc1-deficient DCs, but loss of mTORC2 alone does not have strong effects on DC development. Thus, aberrant activation of mTORC1 in the absence of Tsc1 largely accounts for the defective development. Our results demonstrate that the interplay between Tsc1-Rheb-mTORC1 signaling and Myc-dependent bioenergetic and biosynthetic activities constitutes a key metabolic checkpoint to orchestrate DC development [33].

Although Tsc1 plays a critical role in the development of Flt3L-derived DCs, it is not essential for the in vivo differentiation of GM-CSF-derived DCs [34]. Furthermore, deletion of Tsc1 via the CD11c-Cre system does not alter DC development [8], highlighting a context-dependent requirement of Tsc1 in DC development. Using GM-CSF-derived BMDCs, Pan et al. explored how the deletion of Tsc1 affects TLR-mediated activation and function of DCs [34]. Tsc1 deficiency results in increased production and functional activation of DCs.

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**Figure 2** The roles of mTOR signaling in DC development, maturation and function. DC development and homeostasis are under the control of Pten, Tsc1 and Raptor. Tsc1 is also important for DC maturation and cytokine production, whereas Raptor controls IL-10 production in DCs. Although not depicted here, rapamycin treatment affects the development, maturation and cytokine production and functional activation of DCs.
expression of TNFα and IL-6 but decreased IL-12p40 in response to LPS stimulation. Importantly, the absence of Tsc1 markedly impairs the expression of MHC-II as well as CIITA, a crucial transcription factor required for MHC-II expression, in DCs but not macrophages or B cells. Consequently, Tsc1-deficient BMDCs show impaired capacity for antigen presentation and activation of CD4+ T cells *in vitro* and *in vivo*. The defective MHC-II/CIITA expression in the mutant cells is associated with diminished expression of IRF4, and ectopic expression of IRF4 restores the CIITA/MHC-II expression. Moreover, Tsc1-deficient DCs have increased mTORC1 but decreased mTORC2 activity, and silencing of Raptor and rapamycin treatment indicate a crucial role of mTORC1 in regulating IRF4-CIITA-MHC-II expression. Together, these studies establish a crucial role of Tsc1-mTORC1 signaling in mediating IRF4-CIITA-MHC-II expression and DC functions [34].

**Raptor in DC subset homeostasis and immune regulation**

Raptor is the signature component of mTORC1 complex [2]. To study the role of mTORC1 in DC function, Ohtani et al. developed mice with DC-specific deletion of Raptor, D11c-Cre [35]. Loss of Raptor results in expansion of selective DC subsets such as CD8+ splenic cDCs and intestinal CD11c+CD11b+ DCs. Raptor deficiency diminishes the production of IL-10 and accordingly upregulates the expression of CD86 in intestinal CD11c+CD11b+ DCs. Furthermore, mice lacking Raptor in DCs are highly susceptible to dextran sodium sulfate-induced colitis. These results highlight an important role of mTORC1 in orchestrating DC subset homeostasis and anti-inflammatory programs in intestinal DCs [35].

Signaling via mTORC1 is also important for the homeostasis of the Langerhans cell (LC), a specialized DC population in the skin [36]. Using D11c-Cre system to delete Raptor in LCs, Kellersch and Brocker reported that loss of mTORC1 activity results in an age-dependent progressive loss of LCs in the skin. This is ascribed to the impaired survival of Raptor-deficient LCs and more importantly, the increased migration of these cells to leave the skin, an effect associated with altered expression of adhesion and migration molecules. In contrast to a crucial requirement of mTORC1, loss of mTORC2 via deletion of Rictor does not have strong effects on the homeostasis of LCs. Therefore, mTORC1 but not mTORC2 is crucial for the homeostasis of the sentinel DCs in the skin [36].

**SUMMARY**

DCs play a central role in directing the decision between immunity and tolerance and the outcome of immune responses. Although mTOR signaling is best characterized in T cells in the immune system, emerging evidence highlights the importance of mTOR in the regulation of various aspects of DC biology, including the development, homeostasis, maturation and function. These exciting advances have benefited from both pharmacological and genetic approaches to modulate mTOR activation. An important future direction is to develop and employ more advanced strategies such as second-generation mTOR inhibitors and spatially and temporally-controlled genetic deletion systems, to further dissect the functional impacts and mechanistic basis of mTOR signaling in DCs. Given the central roles of mTOR in integrating cellular and environmental cues, how mTOR is modulated by upstream signals in DCs, the key sentinel cell in the immune system, warrants further investigation. These studies may lead to the identification of innovative targets and strategies to modulate DC function for therapeutic intervention of immune-mediated disorders.

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