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RESEARCH HIGHLIGHT

Rictor beyond the TORC: linking the proliferation, migration and FcεRI-mediated degranulation of human mast cells

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Rictor is a cytosolic protein that was originally recognized as a specific component of the mammalian target of rapamycin (mTOR) complex 2 (mTORC2). This complex integrates nutrient- and growth factor-induced signaling cascades to regulate cell proliferation and metabolism. An increasing body of evidence however shows that rictor may also function independently of mTORC2 through association with other proteins and complexes. Recent studies on mast cells demonstrated that in the context of mTORC2 rictor positively regulates proliferation of immature and migration of mature mast cells whereas by itself rictor independently functions as a molecular relay that sets the sensitivity of high affinity receptor for IgE (FcεRI) for activating mast cell degranulation. These novel findings suggest that rictor is a multifunctional protein that plays a role in synchronization of multiple cellular functions in mast cells.

Keywords: Mast Cells; IgE; FcεRI; rictor; mTORC; F-actin; SCF

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Introduction

Rapamycin-insensitive companion of mammalian target of rapamycin (rictor) associates with a variety of proteins and molecular complexes. The best recognized is the association with the mammalian target of rapamycin (mTOR). MTOR associates with either rictor to form mTOR complex 2 (mTORC2) [1] or with a regulatory-associated protein of mTOR (raptor) to form mTOR complex 1 (mTORC1) [2]. The ratio of mTORC2 to mTORC1 is likely determined by the relative intracellular concentrations of rictor and raptor with each competing for binding with mTOR [1]. Apart from rictor or raptor, both complexes contain other partners. Some of these partners associate with only one of the complexes (mTORC2: mSIN, Protor; mTORC1: PRAS40) and some with both of them (mLST8, Deptor, TTI1/TEL2).

The mTORCs affects multiple cellular functions that are associated with cell metabolism, growth/proliferation, survival and energy. A dysregulation of mTORC functions leads to disruption of cell homeostasis and a wide spectrum of pathological events. The mTORC components and indicia of their signaling are upregulated in multiple cancers and as such could also be considered as prognostic markers [3-5]. Rictor expression is required for cancer development [6], cancer cell cycle progression [7], promotion of cancer cell growth and motility [8]. Its ectopic overexpression can also cause a neoplastic transformation [9].

A variety of receptors are known to harness mTORC signaling. However, how such signaling affects cellular functions induced by individual receptors is less clear. Recent studies have already indicated that individual components of mTORCs may exert functions independently

of mTORC-regulated functions. As such, these components may transduce an additional receptor-specific signal and cell response apart from the signaling and pleiotropic actions associated with mTORC itself.

MTORC dependence and independence

The signaling initiated by the mTORCs emanates from the kinase activity of mTOR. MTOR is a serine/threonine kinase whose activity is regulated through phosphorylation of mTOR [10,11] and binding to its molecular partners [12]. Phosphorylation of these partners also affects mTOR kinase activity [13-15]. The mTOR kinase activity can be monitored by phosphorylation of its downstream signaling substrates, p70 S6 kinase (S6K) and Akt. MTOR kinase activity within mTORC1 is responsible for p70 S6K(Thr389) [16-19] and within mTORC2 for Akt(Ser473) phosphorylation [20-23]. Ablation of mTOR kinase activity with specific inhibitors also ablates phosphorylation of these sites [24-26].

However, the use of specific inhibitors of mTOR kinase activity together with down regulation or ablation of mTOR, raptor or rictor expression have indicated discrepancies between the mTORC kinase activity and downstream phosphorylation of mTORC targets namely, p70 S6K(Thr389) and Akt(Ser473) [27,28]. This discrepancy was especially true for mTORC2 where rictor has been reported to act independently of this complex and/or being part of molecular complexes that do not contain mTOR [27,29-33].

MTORCs in mast cells

Mast cells are immune cells that develop from hematopoietic CD13/CD34$^+$/CD117 (KIT)$^+$ bone marrow stem cell progenitors. These progenitors migrate to target peripheral tissues where they expand, differentiate and mature [34]. Expression of mTORC components mTOR, raptor and rictor is highly upregulated in human mast cell progenitors and even increases during the expansion phase of mast cell development [25]. Towards completion of mast cell differentiation and upon their maturation, expression of all these components is largely down regulated. This is particularly true for rictor whose expression is markedly diminished in terminally differentiated mature human mast cells [25]. In contrast, the expression and activity of these components as well as their downstream signaling targets is substantially enhanced in neoplastic human mast cells [25,35].

Terminally differentiated human mast cells are non-dividing and contribute to host defense through release of inflammatory mediators [36]. A variety of mast cell receptors initiate similar early signaling events but diverse effector functions. This is well illustrated for the high affinity receptor for IgE (FcεRI), stem cell factor (SCF) receptor CD117 (KIT), and G protein-coupled receptors (GPCRs) for prostaglandin (PG) E_2. All these receptors trigger signaling events that converge to enable mobilization of intracellular calcium and filamentous (F)-actin rearrangement [37-41]. Low concentrations of stimulants for these receptors are chemotactic and induce mast cell migration. High concentrations of these stimulants induce *de novo* production of inflammatory mediators [42] albeit responsiveness to some of these stimuli, such as PGE_2 in human mast cells, may differ substantially [43]. However, only FcεRI stimulants at higher concentrations can initiate a rapid (anaphylactic) release of preformed granules that contain a large amount of biologically active products such as histamine, heparin and proteases. This anaphylactic release, also called degranulation or anaphylactic degranulation, is not triggered by SCF/KIT- or PGE_2/GPCRs-mediated stimulation [44-46].

All three types of these receptors, however, activate mTORCs and their downstream signaling targets in mast cells [25,35,47]. Inhibition of mTORC1 by rapamycin [35], mTORC1/mTORC2 by a highly-specific inhibitor of mTOR kinase activity Torin1 [25], or even down regulation of mTOR expression [48] has no effect on FcεRI-mediated mast cell degranulation. However, Torin1 or down regulation of rictor expression inhibits PGE_2/GPCRs-elicited mast cell migration. Because rapamycin or down regulation of raptor expression has no impact on this migration, it is likely that TORC2 regulates this process [47]. Mast cell migration elicited through SCF/KIT-mediated stimulation is, however, controlled, at least partially, by mTORC1 because rapamycin partially inhibits this migration [35]. Together, these data show that mTORCs play a role in migratory performance of not only mast cells but likely more profoundly in a migratory performance of neoplastic mast cells and developing mast cell progenitors where mTORC components are upregulated.

Apart from migration, mTORCs play an important role in mast cell survival and proliferation of their progenitors or neoplastic counterparts and, similarly to migration, both complexes differentially regulate these processes [25,49]. It was shown that inhibition of mTORC1/mTORC2 by Torin1 inhibits proliferation of neoplastic human mast cells and mast cell progenitors much efficiently than mTORC1 inhibitor rapamycin [25]. This indicates that both complexes control the proliferation. However, the mTORC1-mediated inhibition of proliferation is caused by a compromised cell survival rather than proliferation because down regulation of raptor expression was found to significantly decrease survival of human mast cells [25]. On the other hand, down regulation of rictor expression was found to have no impact on human mast cell survival but inhibition of proliferation [25]. This shows that rictor controls human mast cell proliferation

without affecting their viability. Such the mode of regulation may then explain why rictor expression in long-lived terminally differentiated and non-dividing human mast cells can be nearly ablated while expression of raptor which is indispensable for human mast cell survival remains at low but steadily expressed levels after the expansion phase of the developing mast cell progenitors [25].

F-actin, mTORC2 and rictor in mast cell degranulation

SCF is a mast cell growth, differentiation and survival factor. At low concentrations it is a chemoattractant that allows mast cell progenitors to migrate to peripheral tissues. In these tissues the levels of SCF, soluble or membrane-bound, varies. Enhanced concentrations of SCF support differentiation and maturation of infiltrated mast cell progenitors but sustained exposure to high SCF concentrations also down regulate responsiveness of mature mast cells to stimulation via FcεRI [50,51]. This unexpected phenomenon was related to down regulation of Hck expression and alterations in F-actin rearrangement following chronic exposure of mast cells to elevated concentrations of SCF prior to activation via FcεRI. Chronic exposure to IL-33 also made cells less responsive to FcεRI challenge [52]. Interestingly, this IL-33-induced hypo-responsiveness is similarly linked to alterations in F-actin rearrangement.

F-actin rearrangement, a process of actin de-polymerization and re-polymerization, regulates mast cell degranulation. The underlying mechanisms proposed was that F-actin rearrangement may either facilitate or prevent granule extrusion [39,41,53-55]. F-actin rearrangement is controlled by a number of signaling molecules, including rictor. Therefore the possibility existed that rictor might also regulate F-actin rearrangement in a way that would also affect mast cell degranulation. Although initial studies showed that inhibition of mTORCs by rapamycin (mTORC1 inhibitor) or by a highly specific mTOR kinase activity inhibitor Torin1 (mTORC1/mTORC2 inhibitor) [24] had no effect on FcεRI-induced mast cell degranulation, a recent study on human mast cells however demonstrated that rictor regulates in a more subtle way the sensitivity setting of FcεRI for induction of mast cell degranulation [48]. This sensitivity is set by the expression level of rictor in the cells. Increased expression of rictor makes FcεRI refractory to induction of degranulation by low concentrations of a stimulant. Decreasing the rictor level in the cells then decreases the concentration of the stimulant at which FcεRI can trigger critical signaling events leading to degranulation namely, mobilization of intracellular calcium and F-actin rearrangement. Surprisingly, these events and ensuing degranulation are not affected by rictor expression when mast cells are stimulated by C3a, a ligand of GPCR C3 receptor (C3R), or thapsigargin which induces receptor-independent and calcium-dependent mast cell degranulation. These data suggested that the rictor's mode of regulation in the FcεRI-stimulated cells differs from these other stimulants because F-actin rearrangement in GPCR C3R-stimulated cells is not affected by down regulation of rictor expression. In support of this is that downregulation of F-actin rearrangement by mTORC1/mTORC2 inhibitor Torin1 in PGE_2/GPCRs-stimulated mast cells does not make the cells degranulate either [47]. Therefore, regulation of F-actin rearrangement on its own through mTORCs and by virtue of that by rictor within mTORC2 is sufficient to affect mast cell migration but not FcεRI-induced degranulation.

The finding that rictor did not affect thapsigargin-induced mast cell degranulation, a stimulant that mobilizes calcium directly, indicated that rictor affects signaling events that precede the calcium mobilization [48]. Indeed, decreasing the expression of rictor in human mast cells allowed FcεRI to more efficiently initiate phosphorylation of adaptor protein LAT1 (at Tyr171) and the subsequent phosphorylation of PLCγ_1 (at Tyr783) which are necessary for induction of further signals for calcium mobilization and cell activation [56]. Accordingly, more efficient phosphorylation of LAT1(Tyr171) and PLCγ_1(Tyr783) was found to also translate to a more efficient phosphorylation of a surrogate marker of PI3K activity Akt(Thr308) [48]. Meanwhile, the enhanced phosphorylation of Akt(Thr308) was not followed by an enhancement of Akt(Ser473) phosphorylation, a surrogate marker of mTORC2 activity. This may indicate that either FcεRI-elicited mTORC2-dependent Akt(Ser473) phosphorylation occurs independently of a rictor-controlled PI3K-dependent phosphorylation or that enhanced PI3K activity increases the activity of mTORC2 to compensate for reduced formation of the mTORC2 due to decreased rictor expression. This compensation may then maintain the same levels of Akt(Ser473) phosphorylation as in human mast cells not affected by the rictor decrease. The latter scenario could be supported by another finding that showed the activity of mTORC1-regulated p70S6K, as determined by phosphorylation of its substrate S6 ribosomal protein(S6RP)(Ser240/244), was enhanced when rictor expression was down regulated in human mast cells [48]. This enhancement might be a consequence of a competition between raptor and rictor for mTOR [1]. If so, more mTORC1 may be formed at the expense of mTORC2 in the cells with decreased rictor expression.

The newly described role of rictor in regulation of FcεRI-signaling and degranulation attributes a novel function for rictor in mast cells (Figure 1). This novel function directly affects the ability of FcεRI to trigger degranulation. The finding that receptor-independent thapsigargin or the

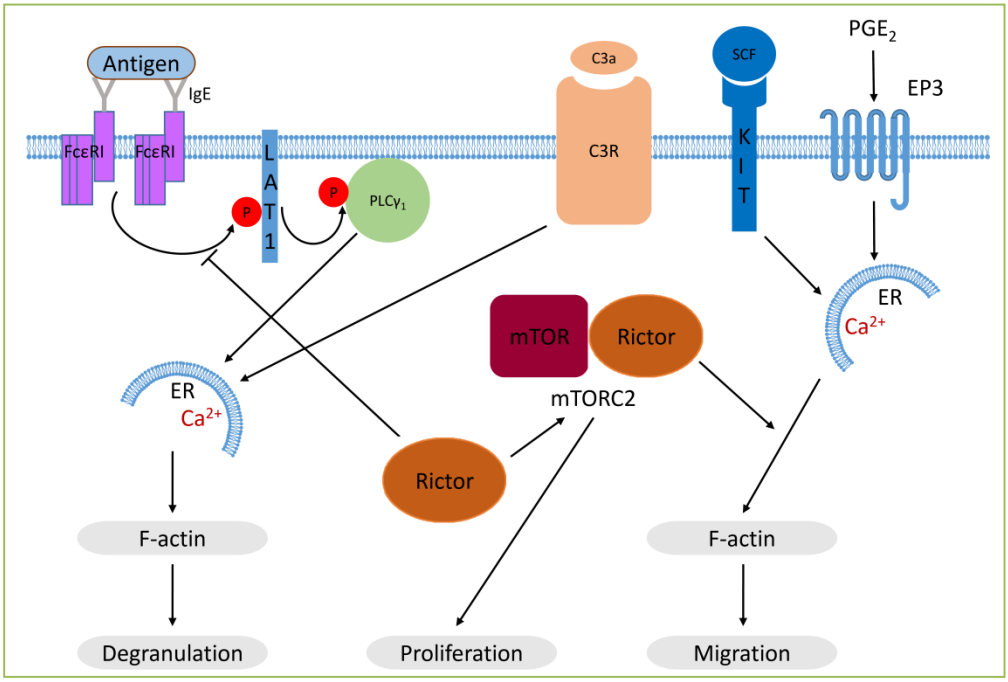

Figure 1. MOTRC2-dependent and -independent role of rictor in stimulated mast cells. Rictor can act independently of mTORC2 by suppressing early signals induced by FcεRI including phosphorylation of LAT1 at Tyr171 and phosphorylation of $PLC_{\gamma 1}$ at Tyr783 and, as a consequence, the mobilization of intracellular calcium from endoplasmatic reticulum (ER), F-actin rearrangement and degranulation. Mobilization of intracellular calcium, F-actin rearrangement and degranulation are not affected by rictor when mast cells are stimulated via C3a/C3R. Rictor within mTORC2 positively regulate F-actin rearrangement, mast cell migration and proliferation. Rictor is presumed to act upstream of LAT1 phosphorylation but the exact mechanism is unknown.

GPCR-dependent C3R-triggered degranulation is not affected by rictor shows that rictor does not generally affect degranulation *per se* and is receptor-specific. This specificity is likely governed by the point of intersection of rictor with FcεRI-induced signaling pathways. Rictor certainly regulates LAT1(Tyr171) phosphorylation. Whether this regulation is a consequence of a regulation that occurs prior to LAT1 phosphorylation is not yet known. It would be expected that the closer the point of intervention to immediate events following FcεRI engagement that are not employed by other receptors the less likely these receptors would be impacted by rictor with regard to mast cell degranulation.

Rictor's orchestration of mast cell degranulation, migration and proliferation

The signaling machinery associated with mTORCs and their individual components was found to modulate critical mast cell functions including proliferation, migration and FcεRI-mediated degranulation. In addition, rictor itself was shown be capable of regulating all these functions either independently or within the context of mTORC2. This signaling plasticity makes rictor a powerful tool through which mast cells can simultaneously regulate their proliferation, migration and FcεRI-mediated degranulation. This single mode of regulation enables switching of FcεRI-mediated functional outputs such as proliferation and migration without degranulation in mast cells.

Migration and proliferation is expected to be enhanced in mast cells progenitors during mast cell development to allow proliferation and infiltration in peripheral tissues [57]. In these progenitors, the expression of rictor is high [25]. Such high expression together with high expression of other mTORC components, mTOR, raptor and rictor, drives the cell proliferation and migration. Simultaneously, high expression of rictor independently of mTORC2 makes FcεRI less sensitive to stimulation to prevent activation before the mast cells are fully developed and homed in the peripheral tissues [48]. Once terminally differentiated and residing in target tissues, these cells minimally migrate and do not proliferate. Under these conditions, the mast cells become sensitive to presence of antigens that recognize FcεRI-bound IgE [48]. The expression of rictor in these cells is minimal as compared to their developing progenitors. Such decreased expression limits mast cell migration [47] and proliferation [25] but allows FcεRI to respond to a much lower level of FcεRI stimulation [48].

Future directions

Rictor is primarily recognized for its function within mTORC2. As such its primary role is widely attributed to regulation of cell proliferation, metabolism and cytoskeleton rearrangement. Expression of rictor, however, also affects cell differentiation [58-61] and contributes to neoplastic transformation [9]. Even though the rictor's mode of regulation in these processes is widely attributed to mTORC2, the recent finding that rictor in human mast cells can regulate sensitivity of one receptor (FcεRI) while leaving the sensitivity of another receptor (C3R) intact rises the possibility that rictor can selectively and independently of mTORC2 modulate responsiveness of individual receptors and through this modulation also to regulate the processes like cell differentiation or neoplastic transformation. Therefore, it would be of interest to determine what other receptors are affected by rictor expression in mast cells and other types of cells in terms of their sensitivity to stimulation and ensuing physiological and pathological consequences.

Conclusions

Recent studies of mTORCs and their components on mast cells revealed that one component, rictor, was identified as the molecule that in addition to its involvement in mTORC2-regulated cellular functions as widely described in other cell systems can also selectively act independently at early stages of FcεRI-mediated cell activation. This unexpected function shows that rictor not only plays a role at late stages of cell activation via mTORC2 but also at early stages of cell activation. With this regulatory potential, rictor may orchestrate responses of mast cells to external stimuli with other cellular functions such as proliferation, migration and degranulation. Understanding the mechanisms how rictor operates and can be modulated in the segregation of these diverse functions may help identify novel therapeutic targets for diseases associated with dysregulated mast cell activation and mast cell proliferative disorders.

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Conflict of interests

Daniel Smrz and Pavla Taborska declare no financial or commercial conflicts of interest. Jirina Bartunkova is a shareholder of biotech company Sotio developing cell-based immune therapies.

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