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Review Article

Regulation of Ras signal transduction during T cell development and activation

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Abstract: T cell receptor-induced activation of the Ras signaling pathway is essential for T cell development, proliferation and differentiation. Given the central role of Ras in T cell biology its activation must be tightly regulated. However, precisely how Ras activation is controlled in T cells is not completely understood. In this review, we provide a summary of the known factors and mechanisms involved in positive and negative regulation of Ras activation in the T cell lineage.

Keywords: T cells, T cell development, T cell activation, T cell receptor, signal transduction, Ras, Ras GTPase-activating proteins, Ras guanine nucleotide exchange factors

Introduction

The small, membrane-tethered GTPase Ras is an oncogene of critical importance to a wide variety of cellular outcomes, including growth, differentiation, activation, apoptosis, and motility [1]. Ras acts as a molecular switch, active when bound to GTP, and inactive when bound to GDP [2]. Ras acts downstream of many different receptors, including growth factor receptors and the T Cell Receptor (TCR). Ras transmits signals through the Raf kinases, which bind to Ras-GTP and trigger the Mitogen Activated Protein Kinase (MAPK) cascade, which culminates in the activation of the MAPK Extracellular-signal Regulated Kinases 1 and 2 (ERK1/2). In turn, MAPKs phosphorylate multiple protein targets, including the transcription factors Jun and Fos that dimerize to form the AP1 transcription factor that drives different downstream cellular responses [3-5]. Ras is also known to activate Phosphoinositide 3-Kinase (PI3K) independently of the ERK/MAPK pathway [6].

There are three classical Ras isoforms, including H-Ras, N-Ras, and K-Ras (which is expressed as the splice variants K-Ras4A and K-Ras4B), which are differentially expressed in separate microdomains within cells [1, 7]. The Ras isoforms are identical in amino acid sequence at their N-terminal Switch I and Switch II domains, which mediate interactions with downstream targets [8]. The only substantial variation in sequence between isoforms is located in the C-terminal regions that contain sites of post-translational modification, which control subcellular localization [8]. In many cases, these isoforms have been found to be non-redundant, and specific Ras isoforms are observed to be critical for cell function in different cell types [1, 9]. Furthermore, K-ras4B has been shown to be essential for normal embryonic development, which distinguishes it from other Ras isoforms [10-13]. T cells express mostly N-Ras and low amounts of K-Ras [14]. N-Ras appears to be the most important Ras isoform in T cells, as TCR stimulation primarily activates N-Ras [9, 14]. In addition, N-Ras mutations are found more often than mutations of other Ras isoforms in T cell leukemias [7].

Activation of Ras in response to TCR stimulation was first demonstrated in human peripheral blood T cells [15]. Subsequently, Ras-MAPK signaling was shown to be of critical importance during T cell development in the thymus. There are three major checkpoints in T cell development, the first of which is known as the TCRβ selection checkpoint. At this checkpoint CD4-

CD8- double negative (DN) thymocytes that successfully rearrange the TCRβ chain gene and express a functional pre-TCR are induced to proliferate and differentiate into CD4+ CD8+ double positive (DP) thymocytes [16]. Constitutively active and dominant-negative forms of the MAPK kinase (MAPKK) Mek1, which lies immediately upstream of ERK in the Ras-MAPK pathway, were shown to enhance or inhibit the transition of DN to DP thymocytes, respectively [17]. In addition, DN to DP transition was found to be blocked in ERK1/2-deficient thymocytes [18]. These findings suggest that the Ras-MAPK cascade is critical for pre-TCR signaling.

The second checkpoint in T cell development occurs when DP cells successfully rearrange the TCRα chain, and express a mature TCRaβ. Cells that express a TCRaβ that can recognize self-MHC molecules receive survival signals, in a process known as positive selection [16]. In this step, DP thymocytes lose expression of either CD4 or CD8 depending upon whether their TCR has preference for MHC Class I or Class II respectively, and mature into single positive (SP) CD4+ or CD8+ T cells. Mice that express dominant-negative or constitutively active forms of Ras or Mek1 show inhibited or enhanced positive selection, respectively [19, 20]. Furthermore, deletion of ERK1/2 in thymocytes also resulted in a nearly complete block in positive selection [18]. Thus, the Ras-MAPK pathway is essential for positive selection.

The third checkpoint in T cell development is known as negative selection, wherein thymocytes that express a TCR that binds to self-MHC/peptide complexes with high affinity are deleted from the repertoire [21]. Expression of dominant-negative forms of Ras and MEK in T cells, which inhibit Ras-MAPK signaling suggested that this pathway is not required for negative selection [19, 22]. In addition, negative selection proceeds normally in the absence of ERK1 and ERK2, indicating that in contrast to TCRβ selection and positive selection, ERK signaling is dispensable for this process [23]. However, although ERK is not involved in negative selection, the MAPK c-Jun N-terminal Kinase (JNK) is required, which can also be activated by Ras [24-26].

In mature T cells, a plethora of studies performed with mice deficient in different components of the Ras-MAPK pathway and with pharmaceutical inhibitors of the Ras-MAPK pathway have all pointed to its essential function in TCR signal transduction leading to T cell proliferation, differentiation, and function [27, 28]. This is consistent with early findings that anergic T cells that are hyporesponsive to TCR stimulation show blocked activation of Ras-MAPK signaling [29, 30]. Taken together, these results demonstrate the essential role of Ras-MAPK signaling in both development and function of T cells.

Distinct classes of regulatory proteins mediate switching of Ras between inactive and active forms. Ras Guanine-nucleotide Exchange Factors (RasGEFs) activate Ras during cellular signal transduction by facilitating the ejection of GDP from the Ras guanine nucleotide-binding pocket. The evacuated pocket is then reoccupied primarily by GTP, owing to its higher concentration in the cytosol, resulting in Ras activation [31]. By contrast, Ras GTPase Activating Proteins (RasGAPs) inactivate Ras by increasing its ability to hydrolyze GTP by up to five orders of magnitude, which results in conversion back to its GDP-bound form [32-34]. The role of different RasGEF and RasGAP family members as regulators of Ras in T cells is discussed below.

RasGEFs in T cells

The first RasGEF to be implicated in Ras activation upon TCR stimulation was mammalian Son of Sevenless (mSOS, Sos1). Sos1 is known to associate with the adapter protein Grb2, which is recruited to the membrane by the tyrosine phosphorylation of the Linker of Activation of T cells (LAT) [35]. Association of Sos1 with LAT in T cells would result in its recruitment to the plasma membrane whereupon it would be juxtaposed to Ras and able to mediate its activation. Sos1-deficient mice die during embryonic development, which has precluded the use of this model to confirm a required role for Sos1 in T cells [36]. More recently, however, a conditional Sos1 allele was generated, and it was determined that deletion of Sos1 in thymocytes in these mice resulted in decreased DN to DP transition, showing that Sos1 plays an important role in pre-TCR signaling [37]. However, both positive and negative thymic selection were normal in these mice, indicating that Sos1 was not required for normal signaling induced

by the mature TCR. While some work has suggested that Sos1 is dispensable for TCR-induced Ras-MAPK activation in peripheral T cells, it is becoming clear that Sos1 plays a more complex role in peripheral T cells, discussed in detail below [38].

Another Ras GEF known to be involved in Ras regulation during TCR signaling is Ras Guanylnucleotide Releasing Protein 1 (RasGRP1). RasGRP1 contains a diacylglycrol (DAG) binding domain which allows its association with the plasma membrane following local activation of phospholipase C gamma (PLCγ) that generates DAG from phosphatidylinositol 4,5 biphosphate (PIP2) [39]. Over-expression of RasGRP1 in T cells results in enhanced Ras-MAPK signaling and IL-2 secretion In T cells in response to stimulation with phorbol ester that mimics DAG. Furthermore, RasGRP1-deficient mice show defective thymocyte DP to SP transition, demonstrating an important function for RasGRP1 in positive selection [40, 41]. Consistent with this, DP thymocytes from RasGRP1-deficient mice are unable to activate the Ras-MAPK pathway in response to TCR triggering or phorbol ester stimulation in vitro. In the periphery, RasGRP1-deficient mice have a marked T cell lymphopenia, most likely due to impaired SP cell maturation [40, 41]. A population of proliferating CD4+ T cells in these animals displays an exhausted phenotype, which is a characteristic of chronic infection [42]. Consistent with this, RasGRP1-deficient mice cleared bacterial and viral challenges at a reduced rate compared to controls.

The extent of overlap between the functions of Sos1 and RasGRP1 in thymic development has been determined in a recent study [43]. In agreement with the earlier demonstrations of roles for Sos1 and RasGRP1 at the TCRβ-selection and positive selection checkpoints respectively, it was determined that Sos1 was expressed primarily in DN thymocytes and that RasGRP1 was expressed most highly in DP cells. The authors confirmed that DN to DP transition was impaired in Sos1-deficient thymocytes. However, additional deletion of RasGRP1 was required to completely block DN to DP transition. By contrast, additional deletion of Sos1 in RasGRP1-deficient thymi did not further impair positive selection observed in the absence of RasGRP1 alone. This work reveals cooperation between RasGRP1 and Sos1 in the activation of Ras at the TCRβ-selection checkpoint. Furthermore, while either Sos1 or RasGRP1-deficient mice showed efficient thymic negative selection, Sos1 and RasGRP1 double-deficient mice were shown to have impaired negative selection. However, in light of the evidence showing that ERKs are dispensable for negative selection, this effect is most likely explained by impaired activation of other downstream effectors of Ras such as JNK.

The interplay between Sos1 and RasGRP1 in peripheral T cells was examined in a separate study [44]. Using Jurkat T cells and primary human CD4+ T cells, the authors determined that upon TCR stimulation RasGRP1 is activated before Sos1. The resulting Ras-GTP is then able to bind to an allosteric pocket of Sos1, which enhances its RasGEF activity. This results in a positive feedback loop, wherein the activity of Sos1 is enhanced by its own product. The authors speculate that this cooperative mechanism between RasGRP1 and Sos1 ensures robust Ras-MAPK signaling even with physiologically low levels of TCR stimulation.

In addition to Sos1 and RasGRP1, other RasGEFs have been suggested to participate in the regulation of Ras during TCR signaling. Another RasGRP family member, RasGRP4, has been shown to play a complementary role to RasGRP1 in thymocyte development [45]. While mice deficient in RasGRP4 alone have no defects in thymic development, mice deficient in both RasGRP1 and RasGRP4 have a more severe block in positive selection than RasGRP1-deficient mice. In addition, partially impaired pre-TCR signaling is evident in RasGRP1/4 double-deficient mice. Consequently, double-deficient mice show severely reduced numbers of mature CD4+ and CD8+ cells in the periphery compared to RasGRP1-deficient mice. Ras GDP-Releasing Factor 2 (RasGRF2) is another RasGEF that is expressed in T cells [46]. However, RasGRF2-deficiency was found to have a minor effect on TCR-stimulated CD4+ and CD8+ T cell proliferation and has been suggested to play a secondary role to RasGRP1 in T cell activation.

RasGAPs in T cells

While the RasGEFs that control TCR signaling in thymic development and in mature T cells have

been relatively well studied, which RasGAPs negatively regulate Ras-MAPK signaling in the T cell lineage is largely unknown. The p120 Ras GAP (RASA1) was the first RasGAP to be discovered [47]. Nine other members of this family have since been identified. In early biochemical studies, RASA1 was suggested to be the RasGAP responsible for downregulation of Ras-MAPK signaling in T cells [15, 48]. However, the assays employed only measured GAP activity of whole cell lysates, and could not distinguish between different RasGAPs that are now known to be expressed in T cells. RASA1 is ubiquitously expressed, and RASA1-deficient mice die in mid-gestation as a consequence of failed patterning of the blood vascular system [49, 50]. With the use of T cell-specific RASA1-deficient mice a relatively subtle role for RASA1 in T cell development was demonstrated. On an MHC Class II-restricted TCR transgenic background, positive selection was enhanced in the absence of RASA1 and this was associated with increased activation of the Ras-MAPK pathway in DP thymocytes [51]. However, increased positive selection was observed neither on an MHC Class I-restricted TCR transgenic background, nor in non-TCR transgenic mice. Therefore, RASA1 is able to function as a negative regulator of Ras during positive selection but only in certain contexts. In peripheral T cells, RASA1 was found to be dispensable as a negative regulator of Ras and T cell activation induced by full agonist MHC-peptide complexes. Nonetheless, T cell-specific RASA1-deficient mice contain substantially fewer naïve T cells, which can be attributed to decreased survival. Unexpectedly, RASA1 is required for normal responsiveness of naïve T cells to the IL-7 cytokine, which promotes their survival in the peripheral immune system [52].

Neurofibromatosis (NF1) is a RasGAP, mutations in the gene of which cause the autosomal dominant disorder neurofibromatosis type I that is characterized by benign and malignant neurofibromas as well as susceptibility to other neoplasms such as myelogenous leukemia [53]. NF1-deficient mice develop cardiovascular and neurological abnormalities and die in utero [54, 55]. Transfer of NF1-deficient hematopoietic cells from these mice into irradiated wild type recipients results in a myeloproliferative disorder [56]. Similar myeloproliferative disorders develop in NF1-deficient adult mice induced to lose NF1 expression de novo [57]. These findings attest to the function of NF1 as an important tumor suppressor in the myeloid lineage. The hematopoetic cell transfer model was also used to study the influence of NF1-deficiency upon T cell development and function. Mice that received NF1-deficient hematopoietic cells showed increased thymic and splenic cellularity and increased basal levels of Ras-GTP in thymocytes compared to mice that received wild type hematopoietic cells [58]. In addition, NF1-deficient thymocytes and mature T cells from these mice showed increased spontaneous proliferation in vitro but impaired proliferation induced in response to TCR stimulation. These findings argue for a function of NF1 as a negative regulator of Ras activation that controls numbers of thymocytes and peripheral T cells in the steady state but also a role for this RasGAP as a positive regulator of T cell activation. Recently, we have investigated the function of NF1 in T cells using a T cell-specific NF1-deficient mouse model (PEL and PDK, unpublished results). Contrary to findings in hematopoietic transfer experiments, we do not observe increased numbers of thymocytes or peripheral T cells in these animals and peripheral T cell activation is also not altered. However, on an MHC class I-restricted TCR transgenic background, loss of NF1 reduces the efficiency of positive selection indicating a positive regulatory role for NF1 in this process. An explanation for the discrepant findings may be a requirement for additional loss of NF1 in hematopoietic cells other than T cells for thymic and splenic hyperplasia and hypo-responsiveness of peripheral T cells to TCR stimulation. For instance, loss of NF1 expression in hematopoietic cells as well as in Schwann cells is required for the development of plexiform neurofibromas in mice [59].

Other Ras-regulating proteins in T cells

In addition to RasGEFs and RasGAPs, another protein, Carabin 1 has been implicated as a direct regulator of Ras in T cells. Carabin 1 was identified as an inhibitor of the NFAT transcription factor pathway in T cells, but also possesses a GAP domain that is present in another family of GTPases with specificity for the Rab small GTPase that is related to, but distinct from Ras [60]. Nonetheless, Carabin 1 was found to decrease steady-state levels of Ras-GTP when over-expressed in the Jurkat T cell line and

knockdown of Carabin 1 expression in primary mouse T cells resulted in increased Ras-GTP levels and increased production of IL-2 in response to antigen stimulation. This suggests that Carabin 1 can function as a RasGTPase in T cells and can regulate Ras signal transduction in this cell type. Surprisingly, however, siRNA knockdown of Carabin 1 in hematopoietic cells had no influence upon T cell development when cells were transferred into irradiated wild-type recipients. Given the established role of Ras at both the TCRβ-selection and positive selection checkpoints of thymic development, this finding is difficult to reconcile with the notion of Carabin 1 as a physiologic regulator of Ras activation in the T cell lineage.

Conclusions

Sos1 and RasGRP1 are the principal RasGEFs involved in the activation of Ras in T cells. In T cell development, Sos1 is required for normal pre-TCR signaling and RasGRP1 is necessary for TCRaβ signaling during positive selection. In addition, cooperation between Sos1 and RasGRP1 in pre-TCR signaling and in negative selection of TCRaβ thymocytes has been identified. In peripheral T cells, RasGRP1 appears to be the most important RasGEF necessary for TCR-induced Ras activation and T cell activation. However, a positive feedback loop of Ras activation in peripheral T cells that involves Sos1 is also recognized. Which RasGAPs mediate Ras inactivation in T cells is less clear. Subtle roles for RASA1 and NF1 in T cell development have been described. Thus, which RasGAPs are the major regulators of Ras activation during T cell development and which RasGAPs control Ras activation in peripheral T cells remains to be determined.

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