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Short Communication

Weak mTORC1 Signaling Strength Controlling T Cell Memory *via* Coupled Transcriptional FOXO1-TCF1-Eomes and Metabolic AMPK-ULK1-ATG7 Pathways

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DESCRIPTION

$CD8^+$ effector T (TE) cells play a critical role in immunity against infections [1]. After an infection, 90%-95% of IL-7RlowCD62LlowKLRG1high Short-Lived Effector Cells (SLECs) undergo cell death in T cell contraction, and the remaining 5%-10% of IL-7RhighCD62LhighKLRG1low memory precursor effector cells (MPECs) differentiates into long-lived memory T (TM) cells that mediate immunity against infection via recall responses [1]. Since the ultimate goal of vaccine development is to generate a larger pool of TM cells, understanding the molecular mechanism that governs T cell memory is of great importance to vaccine or immunotherapy design.

$CD8^+$ TM cells are characterized by two major traits; (a) expression of memory markers IL-7R and CD62L for phenotype differentiation and (b) Fatty Acid Oxidation (FAO) metabolism for generating cell energy, which ensure TM cells with long-term survival and functional recall responses [2]. These two characteristics are controlled by transcriptional FOXO1 (forkhead O transcription factor-1) and metabolic AMP-activated protein kinase pathways (AMPK) [3]. The well-known "progressive linear differentiation" (LCD) model was originally proposed by Sallust in 2000, and later referred to as the distinct strengths of stimuli (antigen (Ag)/cytokine (CK) dose/duration, etc)] model for T cell memory [4-7]. For example, high affinity Ag or T cell receptor (TCR) and high IL-2 with long duration favor TE cell stimulation, whereas low affinity of Ag or TCR and low-dose IL-2 prefer T cell memory formation [6,7]. However, the underlying molecular mechanism controlling T cell memory in the "LCD-TM" model remains unknown.

The mammalian Target of Rapamycin Complex-1 (mTORC1) is a well-known molecular sensor of (to) environmental signals (immune stimuli (three signals: antigen, co-stimulation, and cytokines), insulin, and growth factors) and plays an important role in T cell growth, proliferation, metabolism, and differentiation [8]. In CK-stimulated Phosphatidyllikositol-3-Kinase (PI3K)-AKT-mTORC1 axis, PI3K activates AKT that subsequently activates mTORC1's Raptor [7]. In 2009, Ahmed's group provided the first evidence that inhibition of mTORC1 by Rapamycin (Rapa) treatment promotes $CD8^+$ TM cells [9]. This finding was further supported by TM cell formation by either shRNA-targeted Raptor silencing or Rapa treatment or depletion of the mTORC1 suppressor Tuberous Sclerosis Complex-2 (TSC2) [10-12]. However, the underlying pathway controlling TM cells in the "Rapa inhibition of mTORC1" ("Rapa-TM") model is largely unknown.

It has long been known that three common γ-chain (γc)-family CKs IL-2, IL-7, and IL-15 triggering the same PI3K-pAKT-mTORC1 *via* the same signaling γc [13] distinctly induce short-lived SLECs and long-term MPECs [14,15]. To elucidate the underlying mechanism controlling T cell memory in the "γc-TM" model, we developed IL-2-stimulated TE (IL-2/TE) and IL-7-stimulated TM (IL-7/TM) cells for characterization [16]. We found that, post CK binding, IL-2 and IL-7 stimulate distinct strengths of mTORC1 signaling *via* persistent expression of the IL-2 receptor (IL-2R), leading to IL-2-stimualed strong strength of mTORC1 (IL-2/mTORC1Strong), and transient expression of IL-7R (due to IL-7R internalization), leading to IL-7-stimulated weak strength of mTORC1 (IL-7/mTORC1Weak)[16]. This distinction further leads to forming IL-2/mTORC1Strong-induced TE (IL-2-TE) and IL-7/mTORC1Weak-induced TM (IL-7-TM) cells for our "IL-7-TM" model [16]. This conclusion is supported by some previous evidence that IL-7-activated T cells with constitutive IL-7R differentiated into TE cells rather than TM cells [17,18], while IL-2-stimulated T cells with low expression of IL-2R instead of being TE cells became TM cells [19]. By using state-of-the-art genetic tools, we further demonstrated our in vivo evidence that chicken ovalbumin (OVA)-specific $CD8^+$ T cells with IL-7R-/-derived from engineered OVA-specific TCR transgenic OTI/IL-7R knockout (KO) (OTI/IL-7RKO) mice failed in differentiation into TM cells post OVA-specific recombinant Listeria monocytogenes rLmOVA infection even though having comparable proliferation

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activity compared to CD8⁺ T cells derived from wild-type OTI mice [16]. We also performed *in vitro* and *in vivo* experiments using IL-2/TE and IL-7/TM cells for systematic analysis by Western blotting, multi-colour flow cytometry, confocal and electron microscopy, adoptive T cell transfer, and Seahorse assays [16]. We, for the first time, demonstrated that IL-7/mTORC1Weak signal promotes TM cells *via* coupled transcriptional FOXO1-TCF1 (T cell factor-1)-Eomes (for TM cell phenotypic formation) and metabolic AMPK-ULK1 (Unc-51-like autophagy-activating kinase)-ATG7 (autophagy-related gene-7) for FAO-provided cell energy pathways, whereas IL-2/mTORC1Strong signal induces TE cells *via* activation of transcriptional T-bet (for TE cell phenotypic and functional differentiation) and metabolic hypoxia-inducible factor-1α (HIF-1α for glycolysis-generated cell energy) pathways (Figure 1) [16].

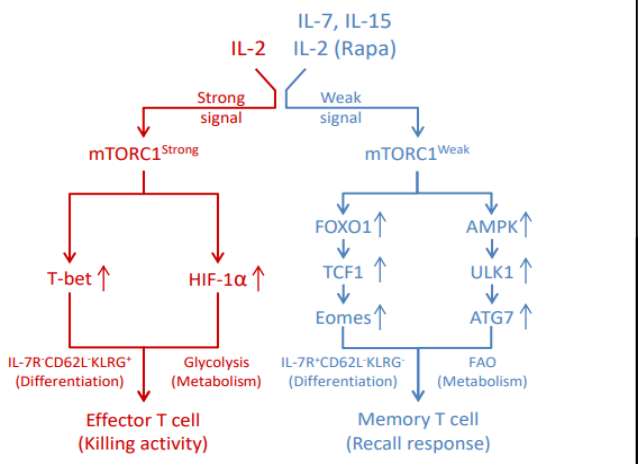

Figure 1: Schematic diagram of distinct strengths of mTORC1 signaling controlling T cell differentiation. **Note:** (▬) IL-2 stimulates strong strength signaling of mTORC1 (mTORC1 Strong), leading to effector T cell differentiation *via* activation of transcriptional T-bet and metabolic HIF-1α pathways; (—) In contrast, IL-7, IL-15, and IL-2+rapamycin [IL-2(Rapa)] stimulate weak strength signaling of mTORC1 (mTORC1 Weak), leading to memory T cell formation via activation of transcriptional FOXO1-TCF1-Eomes and metabolic AMPK-ULK1-ATG7 pathways.

Given that the transcription factor FOXO1 is indispensable in Rictor KO-induced T cell memory [19,20], it is also important to investigate the critical role of e metabolic regulator AMPKα1 in IL-7-stimulated T cell memory. Therefore, we repeated above experiments by using genetically engineered IL-7-stimulated OVA-specific CD8⁺ T cells derived from OVA-specific OTI/AMPKα1KO mice. We demonstrated that AMPKα1 deficiency abolishes the metabolic AMPKα1 pathway and mitochondrial biogenesis, triggers a metabolic switch from FAO to glycolysis and halts its long-term survival and recall responses upon secondary challenge of rLmOVA, even though the transcriptional FOXO1 pathway and TM cell phenotype maintained in IL-7/T(AMPKα-/-) cells derived from OTI/AMPKα1KO mice [16]. Our data thus indicate that the metabolic AMPKα1 pathway is also indispensable for T cell memory. Interestingly, we also found down-regulation of TSC2 and up-regulation of mTORC1 and HIF-1α in IL-7/T (AMPKα1-/-) cells. Therefore, the metabolic switch from FAO to glycolysis in IL-7/T (AMPKα1-/-) cells may be derived from AMPKα1 deficiency-induced loss of TSC2 (an mTORC1 inhibitor), leading to activation of mTORC1 and mTORC1-controlled the transcription factor HIF-1α regulating glycolysis metabolism [21].

To confirm our finding derived from our "IL-7/TM" model, we similarly developed our "IL-15/TM" model for characterization. Interestingly, we observed that IL-15 stimulates transient expression of IL-15R on T cells due to IL-15R internalization post CK binding, leading to IL-15-stimulated IL-15/mTORC1 weak signal-induced IL-15/TM cells [22]. We further demonstrated that IL-15/mTORC1 Weak signal regulates TM cell formation *via* transcriptional FOXO1 and metabolic AMPK pathways, confirming our above finding in the "IL-7/TM" model (Figure 1) [22]. Lastly, to explore the underlying mechanism of the "Rapa-TM" model, we developed our "IL-2/Rapa-TM" model through Rapa treatment to down-regulate mTORC1 [23] for characterization. We found that Rapa-treated mTORC1 weak (IL-2 (Rapa)/mTORC1 Weak) signaling induces TM cell formation *via* activation of FOXO1 and AMPK pathways [23,24].

CONCLUSION

Altogether, our data elucidate the molecular mechanism that distinct strengths of mTORC1 signaling control TE and TM cell differentiation, respectively, via activation of transcriptional T-bet and metabolic HIF-1α pathways and activation of transcriptional FOXO1-TCF1-Eomes and metabolic AMPK-ULK1-TAG7 pathways. In summary, our new finding not only provides the molecular basis of TM cell formation for both "γc-TM" and "LCD-TM" models, but also has great impact on the development of efficient immunotherapies and vaccines for cancer and infectious diseases.

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