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CMTM Kickoff Symposium 2020

Mechanoregulation of T-cell Function through Yap

Nan Cheng [1], Eleni Stampouloglou [1], Gregory L. Szeto [2,3] Xaralabos Varelas [1]

1 Department of Biochemistry, Boston University School of Medicine, Boston, MA, United States

2 Allen Institute for Immunology, Seattle, WA, United States

3 Translational Center for Age-Related Disease and Disparities, University of Maryland Baltimore County, Baltimore, Maryland, United States of America

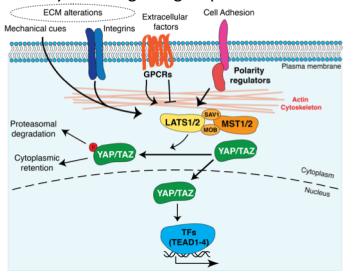
Boston University School of Medicine

Objectives

Although tumor immunotherapy has been developed for various cancers, sustaining T-cell activation and recruitment in immunosuppressive solid tumors remains a major hurdle. T-cell function is modulated by mechanical cues, such as extracellular matrix (ECM) stiffness, but the molecular details by which such signals direct T-cell function are poorly understood. In this study, we investigate the role of Yap, a key mechanotransduction effector, in T-cell function in response to matrix stiffness. We aim to gain a better understanding of mechanoregulation on T-cell function through Yap, which will offer insights for the development of immunotherapies with improved T-cell response.

Background

- The Hippo signaling pathway transduces both internal and external signals to regulate cell proliferation, survival and fate.[1] Yes-associated protein (Yap) and transcriptional coactivator with PDZ-binding motif (Taz) are the major effector in the Hippo pathway which sense mechanic forces to transduce signals that impact gene expression and cytokine signaling responses.[2]



- In T cells, receptor interactions and soluble factors lead to T cell activation, clonal expansion and differentiation to perform a variety of functions central for adaptive immunity.
- T cells receive physical cues in the form of stiffness, shear stress, and tension from surface receptors, which can be transduced into biochemical signals that regulate T-cell responses through activation of ion channels, metabolism, chromatin reprogramming, and gene expression [3].
- **In this study, we investigated the role of Yap in T-cell activity and examine how these signals are regulated by matrix stiffness.**

Results

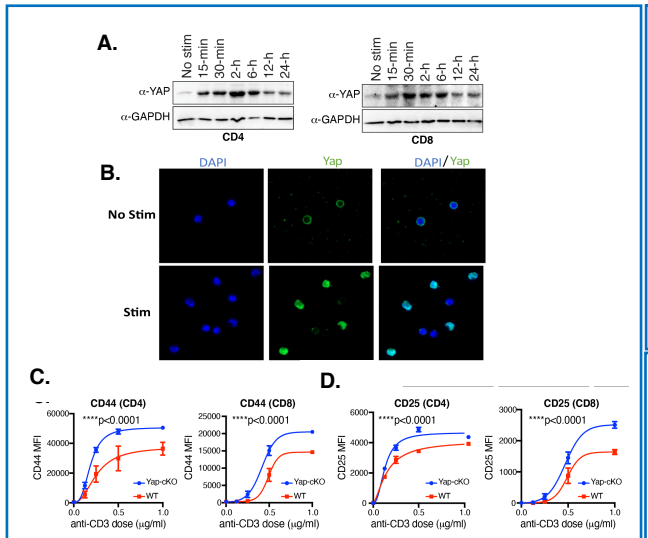


Figure 1. Yap expression is induced upon TCR activation and results in suppression of T-cell activation.
A. Yap expression in both CD4⁺ and CD8⁺ T cells after TCR activation.
B. Immunofluorescent of Yap in non-stimulated and stimulated CD4⁺ T cells.
C. CD25 and CD44 levels in Yap-cKO CD4+ and CD8+ T cells by flow cytometry.

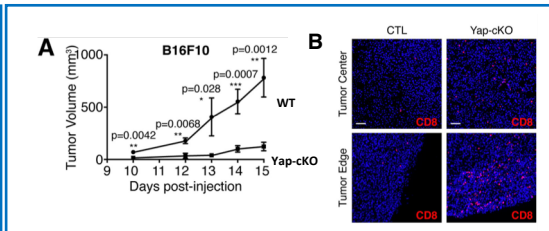


Figure 2. Yap depletion in T cells results in decreased tumor growth and increased tumor infiltration.
A. B16 tumor growth curve in WT and Yap-cKO mice.
B. CD8⁺ T cell infiltration by immunofluorescent staining on B16 tumors.

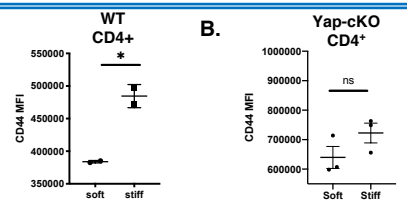


Figure 3. Effects of Matrix stiffness on T-cell response using PDMS culture surface.
A. CD44 expression of WT CD4⁺ T cells cultured on soft and stiff PDMS surfaces after 96hr of activation.
B. CD44 expression of Yap-cKO CD4⁺ T cells cultured on soft and stiff PDMS surfaces after 96hr of activation.

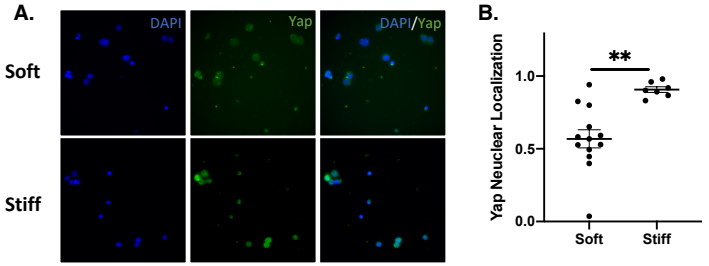


Figure 4. Nuclear localization of Yap in T cells under stiff environment.
A. Representative immunofluorescence images show YAP localization in WT CD4⁺ T cells on soft or stiff PDMS scaffolds.
B. Yap nuclear localization analyzed by colocalization analysis.

Conclusions/Future Direction

- Yap is upregulated in both CD4⁺ and CD8⁺ T cells after TCR activation and exhibits higher nuclear localization.
- Using a T-cell specific Yap conditional knock out mouse model and B16 melanoma tumor model, we observed an inhibitory role of Yap in T-cell activation and anti-tumor immunity, in which Yap-cKO T cells exhibit better activation and increased tumor infiltration[4].
- A Polydimethylsiloxane (PDMS) culture surface was used to construct culturing conditions for T cells with various rigidity. We observed increased T-cell activation in WT cells under stiff environment, whereas Yap-cKO T cells do not show similar responses to stiffness changes.
- Yap shows a higher nuclear localization under stiff microenvironment.
- Overall, our data suggests Yap is playing a key role in modulating T-cell function, and that Yap activity is regulated by matrix stiffness.
- We plan to further investigate the cytoplasmic and transcriptional roles of Yap in T-cell function and how Yap is regulated under different stiffness environments.

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