EPR Analysis of the T-cell Antigen Receptor α Transmembrane Domain

Song, L., Kaur, P. (FSU, Biology and NHMFL); Feng, Y., Lang, M.J. (Vanderbilt U.); Brazin, K.N., Mallis, R.J., Wagner, G., Arthanari, H. and Reinherz, E.L. (Harvard Med. School)

Introduction

T cells and their surface proteins, T-cell antigen receptors (TCRs), perform immune surveillance to prevent or combat infections, cancers, and other diseases. Here, we determined the molecular details of TCR α subunit and its dynamic movements during T cell activation [1]. We first defined the structure of TCR α subunit transmembrane domain and cytoplasmic tail (TCRαTMC) in phospholipid micelles using nuclear magnetic resonance spectroscopy (NMR) [1]. Subsequently, we studied the structural features in liposomes using electron paramagnetic resonance spectroscopy (EPR), permitting membrane immersion depth and intra-helical distance measurements.

Experimental

Experiments at the MagLab involved EPR and spin labeling techniques. The EPR measurements were carried out on a Bruker E680 EPR spectrometer at the MagLab. Liposomes were prepared using the standard extrusion method. Synthetic TCRαTMC wild type and mutant peptides were labeled with MTSL (methyl thiosulfonate spin label). The peptides were dissolved in methanol and subsequently co-dried along with the lipids during the liposome preparation.

Results and Discussion

Using EPR spectroscopy, we measured the relative distances between different segments within TCRαTMC and how deep these segments are immersed in lipids mimicking cell membranes (**Fig.1**). These measurements identified a flexible L-shaped formation of the transmembrane domain of TCRα in the membrane, which undergoes stepwise movements during T cell triggering as demonstrated by functional and mutational studies [1]. Depth measurements of wild type and mutant peptides suggest that positively charged TM residues R251 and K256 project from opposite faces of the helix, with K256 controlling immersion depth (**Fig.1**). Distance and depth measurements of G259L/N261A mutant indicate that this mutation modifies the L-shaped structure and increases peptide helical length. These findings along with the results obtained using other techniques revealed that T cell activation is initiated via a dissociative mechanism, shifting disposition of individual segments to rearrange TCR membrane topology and weaken its association with another T cell surface protein - CD3 [1].

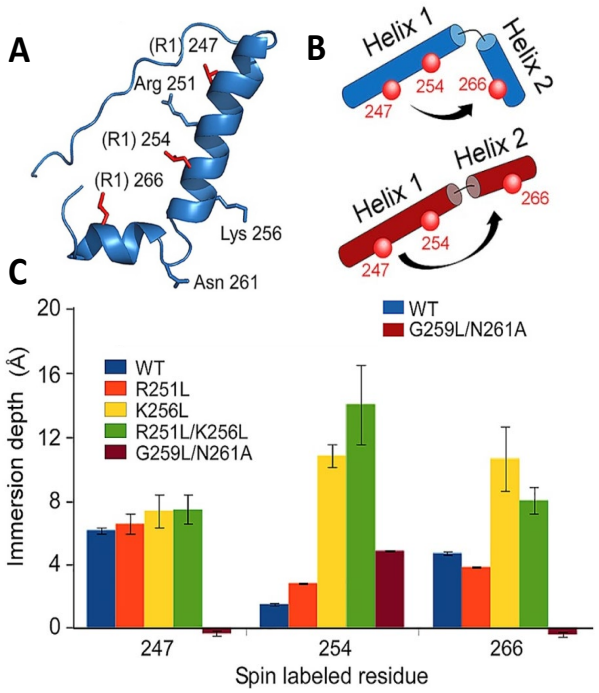

Fig.1 EPR analysis of TCRαTMC. (A). Molecular structure of TCRαTMC. Labeled residues (R1) for EPR studies are highlighted in red. EPR distance (B) and immersion depth (C) measurements of wild type (WT) and mutant TCRαTMC.

Conclusions

This study defined the structural movements within the TCRα transmembrane domain linked to fundamental TCR complex mechanobiology and cell activation. The results show how machinery within T cells responds to outside signals and activates immune responses to attack infected or diseased cells. The findings shed light on developing new therapies regulating how T-cells fight cancers and other diseases.

Acknowledgements

The National High Magnetic Field Laboratory is supported by the National Science Foundation through NSF/DMR-1157490/1644779 and the State of Florida. L.S. acknowledges support from NHMFL UCGP grant #5080. This work is also supported by NIH grants PO1 GM047467 to G.W. and E.L.R., AI037581 to G.W., and R56 AI138489 and R01AI100643 to E.L.R. and M.J.L.

References

[1] Brazin, K.N., *et al.*, Immunity, **49**, 1-13 (2018).