論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	Aalaa Alrahman Mohammad Ahmad Sherif
論文題名 Title	TCR-pMHC complex formation triggers CD3 dynamics (TCR-pMHC複合体形成がCD3ダイナミクスを引き起こす)

論文内容の要旨(Abstract of Thesis)

〔目　的(Objective)〕
The mechanism of T cell activation by peptide-MHC binding (pMHC) has been studied for decades. Upon TCR-pMHC binding, T cells are activated through downstream signaling cascade involving the phosphorylation of the immune receptor tyrosine-based activation motifs (ITAMs) located on the cytoplasmic CD3 long tails. However, the mechanism by which the initial signal propagates from the extracellular TCR-pMHC domains through plasma membrane remains elusive.

〔方法ならびに成績(Methods/Results)〕
We investigated the dynamics of TCRs in both unbound and pMHC-bound states using computational and experimental approaches. Through molecular dynamics simulations, we developed a model referred to as the “drawbridge model,” demonstrating that in the unbound state, the extracellular domain (EC) of the TCR bends over the CD3 chains, with the TCRβ-FG loop acting as a gatekeeper that restricts the mobility of the CD3 complex. Upon pMHC binding, the TCR EC extends, allowing CD3 proteins to move freely, initiating downstream signaling cascades and subsequently activating the T cell.
The flexibility of TCR EC movement is attributed to the connecting peptides (CPs), referred to as the 'hinge region,' which link the TCR extracellular domain (EC) to the transmembrane domain (TM). To further investigate the role of hinge region in T cell stimulation, we introduced mutations in two conserved glycine residues in the TCRβ CP, substituting them with alanine and proline. In MD simulations, these substitutions were predicted to bias the TCR to the activated conformation, with effect of the proline substitution being the strongest. Consistently, the Proline substitution resulted in a significantly higher percentage of activated T cells, whereas the alanine substitution led to only a slight increase in T cell activation compared to the wild type.

〔総　括(Conclusion)〕
The drawbridge molecular model of T cell activation provides a novel explanation of how pMHC binding triggers CD3 dynamics. Consistent with this model, rigidifying the hinge region with a proline mutation enhanced T cell stimulation, suggesting that the TCR hinge region could be a promising target for immunotherapy.

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論文審査の結果の要旨及び担当者

(申請者氏名) Aalaa Alrahman Mohammad Ahmad Sherif

		(職)	氏　　名
論文審査担当者	主　査	大阪大学教授	Daron M. Standley
	副　査	大阪大学教授	Yamamoto Masahiro
	副　査	大阪大学教授	Watanabe Tokiko

論文審査の結果の要旨

Ms. Sherif's thesis project focused on the signaling mechanism of the T cell receptor (TCR). While it is known that T cells become activated when TCRs engage with peptide-MHC (pMHC) complexes having sufficient affinity to form a stable complex, the mechanism by which this information is conveyed to the T cell is controversial. Many models have been proposed, including mechanosensory, kinetic proofreading, clustering etc. In order to investigate this question in an unbiased way, Ms. Sherif made use of recent cryo-EM structures of the complete TCR-CD3 complex and of structures of the TCR ectodomain bound to a pMHC. Her colleague, Floris van Eerden, performed molecular dynamics simulations on a single-membrane system consisting of only the TCR-CD3 complex and of a dual membrane system consisting of the aforementioned TCR-CD3 complex on one membrane and a pMHC embedded in a second membrane. Dr. Van Eerden observed that the dynamics of the CD3 molecules were very different in the two systems. Specifically, in the TCR-CD3 system, the CD3 molecules were tightly coupled to the TCR; in contrast, in the TCR-CD3-pMHC system, the CD3 molecules diffused much more freely, owing to a tilt in the angle of the TCR needed to bind the pMHC. These results implied that the TCR must undergo large-scale movements upon pMHC engagement. When we looked for hinge residues that could facilitate such movement, we found a pair of conserved glycine residues in the TCR beta chain that appeared to act as such a hinge. In order to validate this, Ms. Sherif constructed reporter cells expressing both wildtype and hinge-mutant TCRs by replacing glycine with either alanine or proline. Interestingly, the proline mutant TCRs were hypersensitive within a 2-4 hour window following stimulation. Furthermore, when we carried out molecular dynamics simulations of the mutants, we found that the proline mutants were locked in a conformation close to the TCR-pMHC system. These findings contradicted recent reports that claimed TCRs in resting and activated T cells have nearly identical structures. Interestingly, two months after Ms. Sherif's paper was published, a group in the U.S. demonstrated that the earlier structural work showing a lack of conformational change were due to artifacts in sample preparation resulting from the use of crosslinking and detergents rather than intact membrane. Together Ms. Sherif's publication and the subsequent work from the U.S. demonstrate that TCRs undergo large conformational changes upon pMHC engagement, and that these changes affect the interaction between TCR and CD3. Future work may reveal new molecules that can shift the activation barrier for T cells based on these findings.

This research is worth being granted a doctoral degree (medicine).