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論　文　内　容　の　要　旨

氏　名　（　中村　希　）	
論文題名	Elucidation of the Signal Transduction Mechanism of Homodimeric Plexin B1 Studied by Engineered Dimer-inducing Proteins (Plexin B1の二量体化によるシグナル伝達機構の構造的原理の解明)

論文内容の要旨

Semaphorin-Plexin signaling axis plays a variety of roles in our body including axon guidance, immunomodulation, and angiogenesis. Among them, signaling by Semaphorin 4D (SEMA4D) through its receptor Plexin B1 (PlxnB1) is considered as one of the important targets for drug discovery in the field of cancer and bone metabolism.

Both PlxnB1 and SEMA4D are type-I transmembrane proteins that have a common Sema domain at the N-terminus, followed by two (SEMA4D) or nine (PlxnB1) additional domains in their extracellular region. PlxnB1 has a large cytoplasmic GTPase-activating protein (GAP) domain, which is thought to be activated during the signal transduction by SEMA4D. Crystal structure of the complex between short ectodomain fragments of SEMA4D and PlxnB1 have revealed that two monomeric PlxnB1 Sema domains bind separately to each of the homodimeric Sema domains of SEMA4D, resulting in a 2:2 tetramer structure. This leads to a general hypothesis that PlxnB1 ectodomain dimerization induced by the homodimeric SEMA4D engagement activates its intracellular GAP domain to trigger the signaling, although it is not clear whether a specific dimeric structure is required for the signaling.

If PlxnB1 dimerization at the N-terminal Sema domain alone is sufficient for the signaling, non-physiological dimerizing agents should act as PlxnB1 agonists. To examine this hypothesis, I decided to make use of two cyclic peptides PB1m7 and PB1m6A9 that are known to bind Sema domain of PlxnB1, and constructed various artificial bivalent proteins. I employed the design strategy developed recently in the lab, where the internal sequence of macrocyclic peptides were inserted into loops on the surface of Fc region of human IgG. By inserting the PlxnB1-binding cyclic peptide moieties into the rigid and homodimeric Fc scaffold, I envisioned that each peptide-inserted Fc would induce unique dimeric conformation of PlxnB1 upon the binding to the Sema domain.

The effect of various peptide-inserted Fc on the PlxnB1 signaling was examined by measuring the changes in morphology of PlxnB1-expressing cells. The results showed that some of the PB1m7-inserted Fcs induced “cell collapse” response similar to that triggered by the physiological ligand SEMA4D, suggesting that bivalent PB1m7 can function as PlxnB1 agonist. On the other hand, bivalent form of another cyclic peptide, PB1m6A9, was not only incapable of changing the cell morphology by itself, but rather it suppressed SEMA4D-induced PlxnB1 activation and behaved as an antagonist. These results indicate that simple cross-linking of two PlxnB1 molecules through their Sema domain is insufficient to trigger its activity, but the dimerization must occur through certain conformation (e.g., that induced by either SEMA4D or Fc-PB1m7).

The contrasting effects of the two peptide-Fc fusions in cell-based assay strongly suggest that they bind distinct surface of PlxnB1 Sema domain and induce different dimeric structures. To deduce the conformation of the dimeric PlxnB1 induced by the agonistic and antagonistic peptide-Fc fusions, I determined the crystal structure of PlxnB1 Sema domain bound by monomeric version of PB1m7 or PB1m6A9 fused to a small globular protein uteroglobin, and identified their binding positions. As expected, the binding sites for the two peptides were located at completely different faces on the Sema domain. Based on the peptide docking poses determined above, conformations of the dimeric PlxnB1 cross-linked by agonistic PB1m7-Fc and antagonistic PB1m6A9-Fc were simulated, revealing that the former produced a face-to-face dimer similar to that generated by SEMA4D, while the latter formed a completely different side-to-side dimer. Thus, I succeeded in elucidating the conformation-specific PlxnB1 activation mechanism by showing both signaling competent and incompetent dimer conformations.

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論文審査の結果の要旨及び担当者

氏　名（　中村希　）			
	（職）		氏　名
論文審査担当者	主査	教授	高木 淳一
	副査	教授	山本 亘彦
	副査	教授	中川 敦史
	副査	教授	加藤 貴之

論文審査の結果の要旨

様々な細胞の接着挙動や運動能を制御するセマフォリン-プレキシンシグナリング系においては、一般にホモ2量体を形成しているセマフォリンリガンドがプレキシン受容体に結合することにより細胞上でプレキシンの２量体化が起こることがシグナル伝達の引き金となると考えられているが、シグナル伝達のためにはプレキシン２量体化がどのような形で起こる必要があるのかについて、必ずしもコンセンサスは得られていない。本論文は、セマフォリン4D(Sema4D)によって活性化されるプレキシンB1(PlexinB1)受容体を取り上げ、その活性化型受容体の２量体構造を、（１）PlexnB1結合性環状ペプチドをFcタンパク質に提示した二価の人工タンパク質を用いたシグナリングアッセイと、（２）それらのペプチドとPlexinB1頭部との複合体の結晶構造解析により明らかにしたものである。様々な疾患に係わるSema4D-PlexinB1シグナリング系の構造メカニズムに迫る研究であり、本論文は博士（理学）の学位論文として十分価値あるものと認める。