論文の内容の要旨

論文題目

Study of the regulation of extracellular distribution and signal reception of the Wnt morphogen

（Wnt モルフォゲンの細胞外分布とシグナル受容の制御に関する研究）

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In eumetazoan embryogenesis, positional information plays a central role to establish the basic body plan. Morphogens are defined conceptually as the molecule that gives positional information to cells in a concentration dependent manner. The peptide growth factor Wnt has been postulated to be a morphogen in both vertebrate and invertebrates. However, extracellular behaviours of Wnt ligands, including diffusion, distributions and signalling ranges, and their status on signal reception have remained unclear.

In Chapter I, I show the regulation of distributions and signalling ranges of Wnt by secreted Frizzled-related proteins (sFRPs), which are supposed to negatively modulate Wnt signalling. Although Wnt proteins are thought to diffuse extracellularly and act as morphogens, little is known about the

diffusibility of either Wnts or sFRPs. Here I show that Frzb and Crescent (Cres), members of the sFRP family, have the ability to regulate diffusibilities and signalling areas of Wnt ligands, Wnt8 and Wnt11. I found, using the *Xenopus* embryo, that Wnts do not appear to diffuse effectively, whereas Frzb and Cres spread very widely. Interestingly, Frzb and Cres substantially promoted the diffusion of Wnt8 and Wnt11 through extracellular interactions. Importantly, I show that Wnt8 conveyed by sFRPs can activate canonical Wnt signalling despite the molecular nature of sFRPs as a Wnt inhibitor, suggesting a novel regulatory system for Wnts by sFRPs.

In Chapter II, I aim to elucidate cellular and molecular mechanisms underlying the expansion of the distribution and signalling range of Wnt by sFRPs. I show that the general principle for extracellular distribution of secreted proteins is "binding to cells." by using a newly invented methods, named antibody-based ligand trapping (AbLT). Based on this principle, I demonstrate that most of the secreted Wnt8 and Frzb proteins are bound to the cell surface or extracellular matrices (ECMs), by measuring their dynamics in the extracellular space using fluorescence decay after photoconversion (FDAP) and fluorescence correlation spectroscopy (FCS). Therefore, I address what is the molecular entities that bind and hold Wnt8 and Frzb in the extracellular space. It has been known for many years that heparan sulphate (HS) proteoglycans modulate Wnt distribution and are required for signal reception and transduction. However, their molecular structures and fine distribution on the cell surface have never been considered in the context of Wnt distribution and signal reception. Here I show that newly identified "heparan sulphate nanostructures (HSNSs)" regulate extracellular distributions of Wnt ligands

and act as a preexisting core structure for the formation of Wnt/Dishevelled/GSK3 signalosomes, which is previously reported to be endosomes necesary for signal tranceduction. The data shows that two types of HSNSs have differential specificities of molecular interactions with Wnt and sFRP proteins; that is, Wnt8 co-localises with N-sulpho HSNSs, which is internalized as a core of signalosomes, whereas Frzb co-localises with N-acetyl HSNSs, which relatively stay on the cell surface. Furthermore, with co-expressed Frzb, Wnt8 turns out to preferencially co-localise with N-acetyl HSNSs, similar to Frzb, leading to expansion of Wnt distributions. These data clearly show the integration of ligand distribution and signal reception.

Thus, in this thesis study, which had started from my initial finding of range expansion of Wnt by sFRPs, I have elucidated the regulation of extracellular distribution and signal reception of the Wnt morphogen, as well as the general principle of extracellular distribution of secreted proteins.