論文の内容の要旨

Modulation of Wnt Signaling through Macrocyclic Peptide Wnt3a Ligands
（Wnt3a結合大環状ペプチドを用いたWntシグナル伝達制御）

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The Wnt pathway, both in its canonical and non-canonical signaling cascades, represents one of the best-studied mechanisms underpinning embryonic development and adult homeostasis. Although able to occur through diverse mechanisms, in its most-studied form, canonical Wnt signaling, Wnt protein ligands engage cell-surface Frizzled receptors initiating a signal transduction cascade that ultimately increases intracellular β-catenin concentration and thereby alters the expression of diverse genes (Figure 1). This mechanism involves a variety of proteins and their complexes, many of which have been targeted for therapeutic development due to their involvement in disease states such as colorectal cancer. However, such approaches have generally avoided direct Wnt ligand targeting due to the relative instability and hydrophobicity of Wnt proteins that can cause them to readily aggregate and lose their biological activity, although such direct Wnt inhibitors have the potential for advantageous specificity profiles compared to other agents.

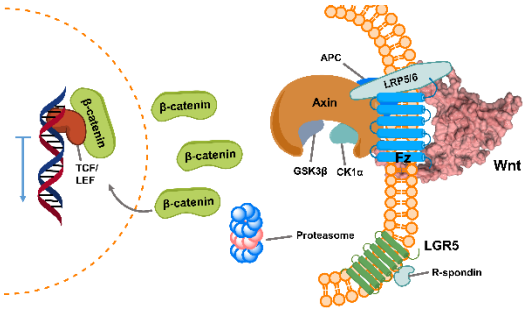

Figure 1 Simplified scheme of canonical Wnt signalling pathway in an active state. Activation of Wnt signalling occurs upon binding of Wnt ligands (shown here as PDB 6AHY) on Fz receptors and oligomerization with LRP 5/6, impeding destruction complex-mediated β-catenin degradation, which can then freely translocate to the nucleus to allow gene expression.

In the present thesis work, we applied the Random, non-standard Peptides Integrated Discovery (RaPID) System with novel selection schemes and comparative sequence analysis in order to identify and develop

de novo cyclic peptide ligands to a Wnt ligand, Wnt3a (Figure 2). In Chapter 2, through the use of a mouse Wnt3a (mWnt3a) protein in complex with a stabilizing protein partner, human Afamin (hAFM), we were able to identify several cyclic peptides capable of binding the target Wnt ligand with no considerable simultaneous binding to its partner, among which a rare peptide hit exhibited potent Wnt signaling inhibition through direct binding to the mWnt3a protein ligand. Further optimization of this molecule through the design of an inhibitor-based library for block mutagenesis and re-selection with the mWnt3a-hAFM complex allowed the development of a peptide with greatly improved binding and signaling inhibition traits, the first of its kind to exhibit such properties by direct binding an extracellular Wnt protein (Figure 3).

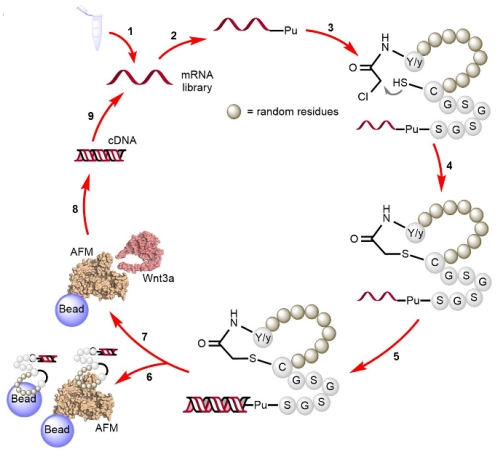

Figure 2 RaPID selection of macrocyclic peptide ligands against mWnt3a-hAFM. A RaPID system-based strategy was designed to target a Wnt-ligand within a protein-stabilized, non-covalent complex, in this case hAFM.

In Chapter 3, taking into consideration the results obtained while targeting a Wnt ligand within a protein-stabilized complex, we targeted the mWnt3a ligand in another form, bound to its natural Frizzled (Fz) receptor, mFz8. Although the presence of a natural binding partner (with therefore highly enhanced binding kinetics) in such a complex was not expected to favor the recovery of peptides that could outcompete the receptor for binding to mWnt3a's active site, we envisioned the process would still allow the identification of peptides with an allosteric effect on signaling activity. Through a similar selection scheme as with the mWnt3a-hAFM complex, we were able to find several peptide binders that showed a higher

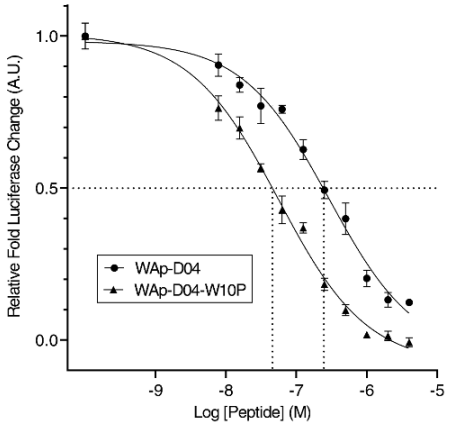

Figure 3 Comparison of the activity of the strongest peptide inhibitors found in this work: WAp-D04 and single W10P mutant. Shown is a sigmoidal fit of luciferase reporter assay results (mean ± SD, n=4) for both. Concentration value corresponding to IC_{50} is marked with dotted lines for comparison.

degree of structural variability than seen before, from which a single lariat peptide was able to considerably inhibit the signaling pathway. Surprisingly, we also identified a different peptide that appeared to heighten Wnt signaling instead of inhibiting it, an effect that we were able to confirm through independent biological assays (Figure 4). Furthermore, these two novel peptide binders to mWnt3a showed structures notably different to the one found by targeting the mWnt3a-hAFM complex, while binding sites and bioactivity effects are expected to occur through different mechanisms.

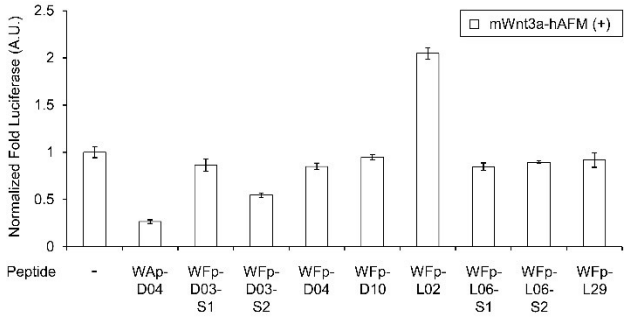

Figure 4 Luciferase reporter assay results of peptides selected against mWnt3a-mFz8 (mean ± SD, n=4). Results show marked inhibitory activity of WFp-D03-S2 and contrasting overactivating activity of WFp-L02. Original peptide WAp-D04 selected against mWnt3a-hAFM is also shown for comparison.

The results found through this work represent the first report of non-natural, mid-sized binding molecules to mWnt3a exerting a strong signaling modulation effect (both inhibition and stimulation) by directly binding a Wnt ligand. Moreover, the results also highlight the potential for using stabilizing protein partners such as hAFM used in this work as a strategy for targeting insoluble and/or unstable proteins through affinity-based selection schemes, as well as increasing the range of protein targets able to be screened with RaPID approaches.