Dickkopf Report Summary

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Final Report Summary - DICKKOPF (Biophysical and structural studies on Wnt-regulatory complexes of LRP5/6, Dickkopf and Kremen)

Background:
The Wnt signalling system is regarded the major developmental signalling pathway in animals. The morphogenic Wnt proteins are secreted proteins, which orchestrate developmental processes such as body axis formation, organ formation and tissue patterning by binding to receptors on the cell surface. Wnt signalling is subject to a multi-layered system of regulation.

Accordingly, misregulation of Wnt signalling is implicated in developmental defects, degenerative diseases, and cancer. Wnts deliver their signals by binding simultaneously to the extracellular domains of two cell surface receptors called Frizzled and Low-density lipoprotein receptor-related protein (LRP) 5 and 61. Its best described extracellular antagonist Dickkopf (German for "large head") functions by blocking access to LRP5 and -62, 3. Kremen proteins potentiate the outcome of the Dickkopf vs. Wnt competition for LRP6 by enhancing LRP5/6 receptor availability in the absence of Dickkopf and rapid removal of LRP5/6 when Dickkopf is present4, 5.

An additional secreted feedback inhibitor of Wnt is the secreted enzyme Notum, which has been identified in genetic studies6, 7. Its supposed mode of action is the release of heparan proteoglycan carrying glypicans from the cell surface so that they cannot fulfil their task to enrich Wnts at the cell surface.

R-spondin proteins are the sole secreted potentiators of Wnt signalling in vertebrates8, 9. Only recently it was found that they bind strongly to the stem cell surface receptors LGR4, 5 and 69-13 and also to the membrane bound E3 ubiquitin ligases RNF43 and ZNRF314, 15. RNF43 and ZNRF3 have been shown to be feedback inhibitors of Wnt which mark the Wnt receptor Frizzled for degradation. R-spondins interfere with this function by crosslinking LGRs and ZNRF3/RNF43 resulting in inhibition of the latter.

Objectives:
The original scope of this project was to provide by means of structural, biophysical and cell-based studies, a functional, mechanistic insight into the negative regulation of Wnt signalling by the secreted protein Dickkopf (Dkk) and its transmembrane receptor Kremen (Krm). Dictated by the publication of several high impact papers, which provided considerable insight into Wnt-inhibition by Dkk16-18 just before the start of the project, the scope was extended to provide insight also into the positive Wnt regulation by secreted R-spondin (Rspo) proteins and its two transmembrane receptor types ZNRF3/RNF43 and LGR4/5/6 as well as the negative Wnt regulation by the secreted enzyme Notum.

Approach:
We produced functional proteins by expression in mammalian cells, which ensures proper folding and the correct posttranslational modifications such as N-glycosylation and disulfide formation. In a largely parallel effort hundreds of clones were screened for expression in small scale trials, optimized and used for large scale transient expression. The proteins were purified from the cell medium. Their mode of interaction and function was studied by X-ray crystallography in which the proteins are crystallized from an over-saturated solution and the crystals exposed to monochromatic X-rays. Studies on the structure of the proteins and complexes thereof was supplemented by biophysical assays (analytical ultracentrifugation and surface plasmon resonance), cell-based functional assays and enzyme assays.

Results:
I could solve the crystal structure of an unliganded, signalling competent fragment of Rspo2 at high resolution. The construct encompassed the two Furin-like cysteine-rich motifs that are also found in cell surface receptors and other extracellular proteins. They adopt a rod-like structure with a ladder of parallel beta hairpin loops that is stabilized by 8 disulfide bridges. Complex structures with the ectodomains of RNF43 and ZNRF3 together with biophysical experiments revealed that the Furin1 repeat is sufficient to bind to the E3 ligase ectodomain. However, Furin1 is not sufficient to trigger signalling as evident from cell-based functional assays. We could map the binding site of Rspo proteins for LGR receptors to the Furin2 repeat. Our structural data hence supports the model in which Rspo proteins serve to crosslink LGRs and RNF43/ZNRF3 via distinct binding sites into a ternary complex which is then removed from the cell membrane. Interestingly, we found that in the case of ZNRF3 but not RNF43 the E3 ectodomain has a propensity to dimerize that is enhanced by ligand binding. Most of our results on Rspo, LGRs and ZNRF3/RNF43 have been published19.

I have also solved the structure of the human Notum enzyme at high resolution. Comparison of three different crystal forms define structurally flexible elements of the Notum enzyme. The enzyme has a high affinity towards heparin and structural determinants for heparin binding could be identified. We continue to work with collaborators to identify the mechanism of this Wnt inhibitor.

The structure of the Kremen ectodomain was solved in two crystal forms by means of molecular replacement. Using these structures and the published structures of LRP and Dickkopf a low resolution ternary complex could be solved as well. This defines the general architecture of the ternary complex in which Dickkopf is sandwiched between Kremen and LRP. Future experiments will confirm this interface by biophysical assays and study how mutants with a disrupted interface behave in cell-based functional assays.

Conclusion:
Our results provide a mechanistic insight into molecular recognition of Wnt signal regulators at the cell surface. These insights are required to rationalize future approaches of therapeutic intervention in cases of misregulated Wnt signalling as for example in cancer.

References

Related information

Result In Brief

Molecular locks and keys in cancer

Documents and Publications

final1-rspo-signalling-membrane-model.png
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