G-PROTEIN-COUPLED RECEPTORS

Lipidated peptides: a therapeutic tool kit

Although they are remarkably diverse in function, G-protein-coupled receptors (GPCRs) share a highly conserved topology of a seven-transmembrane helical core domain joined by three intracellular loops and three extracellular loops. In the October issue of *Nature Medicine*, Covic *et al.* showed that the attachment of a palmitate group to peptide sequences derived from the third intracellular loop of the GPCRs protease-activated receptor 1 (PAR1) and PAR4 resulted in the production of potent antagonists of receptor signalling. These cell-penetrating lipidated peptides, known as pepducins, can be used as tools to delineate the physiological roles of GPCRs and determine the therapeutic value of blockade of a particular signalling pathway.

The large number of GPCRs and their role in disease have made them attractive drug targets. As most GPCR agonists and antagonists act on the extracellular surface of the receptor, the intracellular surface has not been exploited for therapeutic targets. However, pepducins act as receptor-modulating agents by targeting the intracellular surface of the GPCR.

PAR1 and PAR4 are activated by the protease thrombin, which results in the activation of platelets. The ability to control this signalling cascade would be useful in preventing thrombotic complications associated with heart attacks and stroke. The present study set out to develop and test PAR1- and PAR4-based pepducin antagonists for their ability to block platelet activation and thrombosis *in vivo*.

Soluble peptide ligands of PAR1 and PAR4 were inhibited by their respective pepducins. Pretreatment of human platelets with anti-PAR1 pepducin completely blocked aggregation in response to 3 nM thrombin, a physiological concentration. Aggregation was not blocked in response to 20 nM thrombin, presumably because of the signal from PAR4. However, the anti-PAR4 pepducin inhibited most aggregation in response to 20 nM thrombin, indicating that the anti-PAR4 pepducin also inhibits PAR1 signalling. Infusion of the anti-PAR4 pepducin into mice extended bleeding time and protected against systemic platelet activation, consistent with the phenotype of a Par4-deficient mouse. As expected, the anti-PAR1 pepducin did not have any effect on bleeding time, as mouse platelets lack Par1.

The use of pepducins provides a powerful and general approach to determine the effect of disruption of GPCR signalling. This will be particularly useful for GPCRs for which there are no known extracellular antagonists, or in cases in which genetic deficiency results in embryonic death. In addition, the pepducins themselves could be used as therapeutics. In fact, unlike many small-peptide drugs, such as the platelet-receptor inhibitor eptifibatide, these lipidated peptides have long half-lives. This is probably because they partition to cell membranes, where they become resistant to clearance and attack by proteases.

Melanie Brazil

References and links

ORIGINAL RESEARCH PAPER Covic, L. *et al.* Pepducin-based intervention of thrombin receptor signalling and systemic platelet activation. *Nature Med.* 2002 Oct (doi: 10.1038/nm760)

FURTHER READING Covic, L., Gresser, A. L., Talavera, J., Swift, S. & Kuliopulos, A. Activation and inhibition of G protein-coupled receptors by cell-penetrating, membrane-tethered peptides. *Proc. Natl Acad. Sci. USA* **99**, 643–648 (2002) | Covic, L., Singh, C., Smith, H. & Kuliopulos, A. Role of the PAR4 thrombin receptor in stabilizing platelet-platelet aggregates as revealed by a patient with Hermansky–Pudlak syndrome. *Thromb. Haemost.* **87**, 722–727 (2002)

WEB SITE

Athan Kuliopulos' laboratory: http://www.nemc.org/mcri/Kuliopuloslab/kuliopuloslab.htm

IN BRIEF

BIOTECHNOLOGY

Controlling protein activity with ligand-regulated RNA aptamers.

Vuyisich, M. & Beal, P. A. *Chem. Biol.* **9**, 907–913 (2002)

Aptamers — single-stranded nucleic acids that can fold into intricate globular structures capable of binding proteins with high potency and specificity — have potential both as therapeutics and as tools to probe cellular processes. Vuyisch and Beal describe a general method for discovering aptamers that bind to a target protein, with this binding being regulable by a small molecule. Such ligand-regulated aptamers offer greater temporal control of the targeted protein activity, and should prove particularly useful in defining the function of proteins that are involved in processes in which the timing of events is crucial, such as the cell cycle.

INFLAMMATORY DISEASES

The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis.

McCoy, J. M. *et al. J. Clin. Invest.* **110**, 651–658 (2002)

Prostaglandin E2 — which can act through at least four different G-protein-coupled receptors, EP1–EP4 — has been implicated in rheumatoid arthritis. Mice that lack the EP4 receptor show decreased incidence and severity of disease in a model of rheumatoid arthritis, but this is not the case in mice that lack either EP1, EP2 or EP3, indicating that EP4 might represent a novel and specific therapeutic target.

CHEMICAL-LIBRARY DESIGN

Do structurally similar molecules have similar biological activity?

Martin, Y. C. *et al. J. Med. Chem.* **45**, 4350–4358 (2002)

When designing diverse combinatorial libraries or selecting diverse compounds to add to a screening library, computational chemists often reject compounds that are too similar (as assessed by a computational calculation) to compounds that are already in the library to save resources. Martin *et al.* provide data that indicate that the relationship between computational calculations of compound similarity and biological activity is not as strong as has been previously assumed, which has important implications for strategies for compound acquisition and the design of combinatorial libraries.

ANTICANCER DRUGS

Medulloblastoma growth inhibition by Hedgehog pathway blockade.

Berman, D. M. *et al. Science* **297**, 1559–1561 (2002)

There are no effective treatments for medulloblastoma, the most common malignant brain tumour in children. Using cyclopamine — a known antagonist of a protein involved in the Hedgehog signalling pathway — Berman *et al.* show that the pathway is important for the growth of medulloblastoma cells and is therefore a potential target for therapeutic intervention.