inactive conformation of the helix.

This new type of antagonism is probably not unique to THC and ER-β. There are other examples of nuclear-receptor ligands that act as antagonists, even though they might be smaller than the endogenous agonists. Flutamide, a synthetic androgen-receptor antagonist, is similar in size to testosterone, and does not have a bulky side chain to act as an antagonist. Also, progesterone is smaller than aldosterone, but is a high-affinity antagonist of the mineralocorticoid receptor.

This insight into such non-classical antagonism of nuclear receptors highlights a new possible approach to designing antagonists, in which compounds could be tailored to selectively stabilize inactive conformations of certain nuclear receptors, and the active conformations of others.

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SIGNALLING PATHWAYS

Legless — but still the way forward

The more complex a pathway, the more the output can be fine-tuned by incoming signals. Unfortunately, this also means that there are more opportunities for things to go wrong, as highlighted by the results of three independent studies led by He, Bienz and Basler.

The Wingless (Wg) — or mammalian WNT — pathway transduces signals from the cell surface to the nucleus by preventing the glycogen synthase kinase 3 (GSK3)-mediated phosphorylation of Armadillo/β-catenin. When phosphorylated, Armadillo/β-catenin gets degraded. However, in the absence of phosphorylation — that is, in response to Wg or WNT — Armadillo/β-catenin shuttles to the nucleus to regulate transcription by the T-cell factor, TCF.

He's group studied four key amino-terminal β-catenin residues — Ser33, Ser37, Thr41 and Ser45 — which, when phosphorylated, target this protein for degradation. The residues conform to a consensus GSK3 phosphorylation site, but does GSK3 phosphorylate them all? The authors showed that a separate 'priming' kinase, casein kinase Iα (CKIα), was required to phosphorylate Ser45 before GSK3 could phosphorylate the other residues. In its absence, β-catenin accumulated in the cell. Mutations of axin or the adenomatous polyposis coli (APC) protein, two proteins that allow GSK3 to phosphorylate β-catenin, are already associated with colon cancer, so perhaps it is not surprising that mutations at Ser33, Ser37, Thr41 or Ser45 are associated with colorectal cancer. The requirement of a priming kinase to allow GSK3 to phosphorylate β-catenin also has implications for designing therapeutics for other diseases in which GSK3 is implicated, such as type 2 diabetes.

Two more genes — *legless* and *pygopus* — that function in the Wg/WNT pathway were identified by Basler's group and found to function downstream of Armadillo. *Pygopus* was also identified by Bienz's group. Legless binds to Armadillo, Pygopus binds to Legless, and Armadillo signalling cannot occur without Legless or Pygopus. What, then, do these two proteins do?

Basler's group showed that Armadillo/β-catenin can bind Legless and TCF simultaneously, hinting that Legless and Pygopus might affect Armadillo/β-catenin-mediated transcription. As Pygopus enhanced β-catenin-mediated TCF transcription in tissue-culture cells, this indicates that the principal role of Legless might be to recruit Pygopus to β-catenin in the nucleus to influence gene transcription.

The Legless protein shows three short regions of homology to the human BCL9 protein, which was subsequently shown to be its functional homologue. The *BCL9* gene was originally found juxtaposed to the regulatory elements of an immunoglobulin gene in a cell-line derived from a patient with precursor B-cell acute lymphoblastic leukaemia, in which it was expressed at levels 50-times higher than normal. Although Legless overexpression alone was unable to induce the Wg pathway, higher levels of BCL9 might make cells more sensitive to situations in which the WNT pathway is overactivated, such as the loss of CKIα.

Current anticancer drugs aim to disrupt the β-catenin–TCF complex to inhibit gene transcription, but the authors proposed that targeting the protein–protein interactions between β-catenin, Legless and Pygopus might be an additional possibility. As proof of principle, mutations that prevent Pygopus binding to Legless inhibited the ability of a mutated form of APC to activate the pathway. Furthermore, drugs aimed to interfere with β-catenin–TCF binding must be highly specific to avoid disrupting tumour-suppressive β-catenin–E-cadherin cell–cell adhesions, so targeting the β-catenin–BCL9 interaction instead might be the way forward.

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