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New ligands for defective receptors

Martina Habeck, freelance writer

Rather than trying to mend the faults with defective hormone receptors, scientists are now trying to create hormone analogues that will work more effectively with defective receptors than natural hormones do.

Members of the superfamily of nuclear steroid/hormone receptors have a crucial role in regulating basic physiological functions, such as fatty acid metabolism and reproductive development. The receptor proteins act as transcription factors that control gene function in response to lipophilic molecules (for example, retinoids, vitamin D, thyroid hormone and steroid hormones, such as oestrogen and androgen).

Mutations to nuclear hormone receptors can have serious effects on the metabolic regulation and/or development of an individual. Nuclear hormone receptor-associated genetic disorders include certain forms of diabetes and prostate cancer, rickets and resistance to thyroid hormone (RTH).

Mutant complementing molecules

Many of these disease-associated mutations lie in the ligand-binding pocket of the receptor and prevent the natural ligand from fitting in. 'If you go by the classic lock and key model, the key is normal, but the lock has changed, and now you don't get proper regulation of the genes in response to the ligand,' explains chemist John Koh from the University of Delaware (http://www.udel.edu).

Koh believes that in these cases, disease caused by defective receptors can be treated by making a new key. This idea of finding ligand or hormone analogues that fit better into the mutated binding pocket is not entirely new. For example, Sara Peleg and colleagues at the MD Anderson Cancer Center (http://www.mdanderson.org/) screened known vitamin D analogues that would complement vitamin D receptor mutations associated with rickets [1] (see Fig. 1). Similarly, other investigators have screened thyroid analogues and found that certain ones are more potent than the normal thyroid hormone in patients with RTH [2]. Their research indicated that modified ligands might interact with those mutant receptors differently from the natural hormone and could thus restore function.

But will this research have any practical benefits? The number of individuals that harbour any specific nuclear-receptor mutation is small. 'We are looking at clinical trial-size groups of people,' says Koh. 'For example, in RTH, the world population with any one mutation might be less than 100 people.' It is therefore not clear how compounds that complement one particular receptor mutation can be developed into a marketable drug.

For Peleg, this means that research into hormone/ligand analogues that can restore mutant receptor function is 'an interesting intellectual challenge, but is not likely to lead to practical solutions'. But for Koh, the challenge has only just begun.

A clever approach

Koh points out that 'we are one of the first generations to be able to see at the atomic and molecular level what these genetic defects in nuclear receptors look

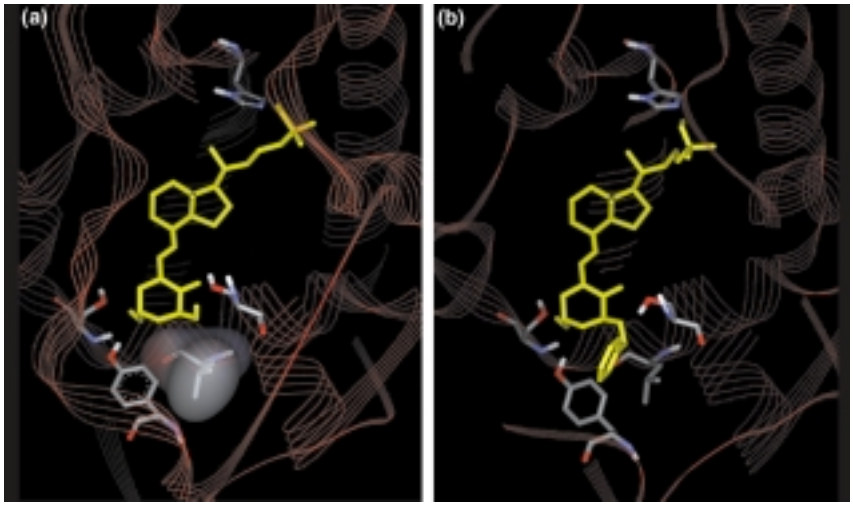

Figure 1. Comparison of the modelled structure of natural vitamin D **(a)** and a vitamin D analogue **(b)** bound to the R274L mutant of the vitamin D receptor. **(a)** As a result of the R274L substitution, an important hydrophilic contact point between the receptor and the natural ligand is lost. There is now a hydrophobic hole within the ligand–receptor interface. **(b)** The designed vitamin D analogue has a hydrophobic group to replace the lost hydrogen bond point with a hydrophobic bond. Figure kindly provided by Steve Swann, Department of Chemistry and Biochemistry, University of Delaware (http://www.udel.edu).

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like, and we have evolved to the level of sophistication now where we may be able to actually do something about it'.

Indeed, crystal structures of the natural thyroid and vitamin D receptor binding to their respective ligands have recently become available [3,4], and according to Koh, there are also unpublished reports of the crystal structures of mutated receptors. Koh's group is using this information to rationally design small-molecule analogues that restore function to defective thyroid and vitamin D receptors [5–7].

All the analogues Koh and colleagues work with are initially designed *in silico*, 'based on what is easy to make from commercially available materials', explains Koh. The compounds are first evaluated by virtual screening (the scientists create a model of the mutated binding pocket and predict how a specific compound will fit in); then, the best candidates are synthesized and tested in cell-based assays.

They did not achieve a 100% success rate, but in the case of a vitamin D receptor mutant, they were able to rapidly identify more potent analogues with higher efficiency using rational molecular design than was obtained from random screening. One of their small-molecule compounds was 526-times more potent than natural vitamin D.

Koh concludes that 'it might not be so difficult to identify compounds that can complement these genetic mutations.' He hopes that this, combined with the fact that the small-molecule compounds are relatively cheap and easy to synthesize, will increase the chances to bring therapeutics to the clinic. 'This may never make any money, but it may have significant value from a humanitarian point of view,' he notes.

Not there yet

Peleg finds Koh's approach interesting and attractive: 'I like the clever thinking here'. However, she wonders whether small-molecule compounds will really do the trick. Throughout the transactivation process, the hormone–receptor complex interacts with other factors, such as co-activators and dimerization partners. During these interactions, the receptor changes conformation and the natural ligand is flexible enough to adapt to these changes. But small-molecule mimics might be too rigid to adjust, and thus important receptor functions could be lost.

Peleg is also concerned that the designer compounds might interact with other nuclear receptors and cause unexpected side effects. During their screen, Koh and colleagues tried to exclude compounds that interact with a membrane-bound vitamin D receptor [8], which has different functions than the nuclear vitamin D receptor associated with rickets. But he agrees that ultimately, 'we need to move into animal models with those compounds. Any applications involving human subjects are certainly many years away'.

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