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Preview

Finding Specificity within a Conserved Interaction Site

Recently developed approaches to generate drugs that regulate hormone-induced gene activation focus on modulating the interaction of nuclear receptors with coactivators. A study by Geistlinger and Guy [1] demonstrates the feasibility of this approach and provides surprising evidence for specificity within the conserved nuclear receptor:coactivator interaction surface.

Many important developmental and physiological processes are regulated by hormones that act through members of the conserved nuclear receptor family [2]. These receptors are intracellular transcription factors that change the expression of hormone-responsive target genes. Due to their involvement in a broad range of common diseases, including breast and prostate cancer, arthritis, obesity, and diabetes, nuclear receptors have advanced to become one of the top targets of basic and pharmaceutical research. Thus far, most drug design efforts have been focused on the development of antagonists, synthetic ligands that compete with natural hormones and block the ability of nuclear receptors to regulate transcription. Although in some cases it has been possible to identify nuclear receptor antagonists that act tissue specifically, generally it has been difficult to separate pathological activities of these receptors in one tissue from their beneficial effects in other tissues. Thus, while many antagonists are valuable therapeutic agents in the treatment of hormone-dependent diseases, their use is often associated with unwanted side effects. Hence, the identification of alternative strategies to regulate the transcriptional activity of nuclear receptors has become of increasing interest.

The Nuclear Receptor:Coregulator Interaction Site as a New Drug Target

Recent structural and functional studies have revealed the molecular mechanisms by which nuclear receptors regulate transcription [3]. The breakthrough came with the discovery that hormone binding regulates the interaction of nuclear receptors with coregulators that increase (coactivators) or decrease (corepressors) the expression of hormone-responsive genes. Although many of these coregulators are structurally and functionally diverse, their interaction with nuclear receptors is often mediated by short amphipathic α helices that, in the case of coactivators, contain a conserved LxxLL sequence motif (L is leucine, x is any amino acid) [4]. Crystallographic analyses showed that the hydrophobic leucine residues in this motif interact with a shallow, solvent-exposed hydrophobic groove in the nuclear receptor ligand binding domain (LBD) (Figure 1) [5–8]. The identification of an α helix that changes the conformation of this groove in response to hormone binding has revealed the molecular mechanism by which these interactions are regulated by hormones [5]. Biochemical studies have demonstrated the feasibility of blocking the interaction between nuclear receptors and coactivators with the help of small peptides containing the LxxLL interaction motif [8–11]. These results suggest a possible hormone-independent mechanism for regulating the transcriptional activity of nuclear receptors and move the interaction of coregulators with the hydrophobic groove into the spotlight for drug design.

The Specificity of Nuclear Receptor:Coactivator Interactions Relies on Receptor-Specific Surfaces

To be useful therapeutic agents, inhibitors that block the interaction between nuclear receptors and coregulators need to be both receptor and coregulator specific. The interaction surfaces between nuclear receptors and coactivators, however, are highly conserved (Figure 2), and it is not obvious how these interactions could be disrupted selectively.

Reporter analyses in cultured mammalian cells and "knock out" studies in mice have revealed that nuclear receptor:coactivator interactions are, at least to some degree, selective [12–15]. Comparative structural and sequence analyses showed that while the coactivator LxxLL motif and the interior of the nuclear receptor hydrophobic groove are highly conserved, the sequences adjacent to the LxxLL motif and the structure of the rim of the groove are variable. Peptide competition experiments and site-directed mutagenesis approaches exploring these differences confirmed their ability in modulating the affinity of nuclear receptor:coactivator interactions [8–11]. Other results suggested that some nuclear receptor:coactivator interactions are stabilized by additional interaction surfaces [16–18]. While these results were promising, they indicated that it might be difficult to find a general strategy to develop small molecules that disrupt these interactions specifically.

Conserved Nuclear Receptor:Coactivator Interaction Surfaces Can Be Disrupted Receptor Specifically

The recently published study by Geislinger and Guy [1] brings an unexpected turn to this story by demonstrating the feasibility of developing selective inhibitors for even the conserved LxxLL:hydrophobic groove interaction itself. Guided by in silico docking experiments using existing crystal structures of human estrogen (hER) and thyroid hormone receptor (hTR) coactivator complexes [7, 8], Geislinger and Guy produced a library of 87 potential proteomimetics of a particular coactivator LxxLL interaction motif in which conserved leucine residues are individually replaced with nonnatural amino acids. Fluorescence polarization equilibrium competition assays using these compounds revealed that 71 of the 87 compounds bind hER and hTR with affinity equal to or higher than the original LxxLL interaction motif. An astonishing finding was that many of these compounds are selective for particular nuclear receptors: 12 of the identified compounds were between 10- and 600-fold selective for

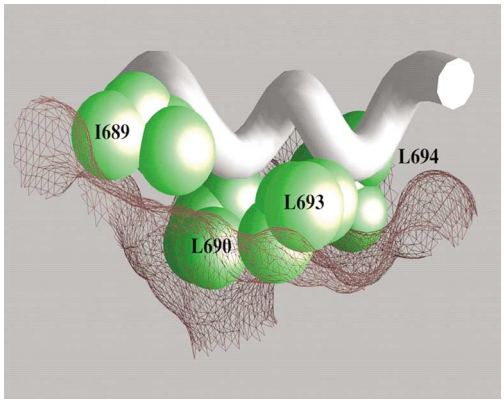

Figure 1. Interaction of the Nuclear Receptor Hydrophobic Groove with a Coactivator LxxLL Motif

Shown is the interaction of the hydrophobic groove of the human thyroid hormone receptor (hTRβ) with an ILxxLL motif of the p160 coactivator GRIP1 [8]. The hydrophobic residues of the ILxxL motif are represented in green, with the Cα peptide chain in white. The three conserved leucine residues tightly fit into the pockets of the hydrophobic groove, which is depicted as mesh.

binding to hERα in preference to hERβ or hTRβ, in addition to one hERβ- and one hTRβ-selective compound [1].

These results demonstrate that the differences in the shape and charge distribution in the conserved, hydrophobic groove of nuclear receptors are sufficient to allow the development of specific inhibitors and open the possibility of a general approach to the development of selective nuclear receptor-regulating drugs. It is possible that these variations in the fine structure of these conserved hydrophobic grooves are evolutionary fluctuations that do not affect the general shape and character of the groove and the binding of coregulators. However, it is also possible that these differences allow nuclear receptors to discriminate between different coactivators and that the contribution of the hydrophobic groove itself in mediating coactivator selectivity has been underappreciated. Much still remains to be learned about the receptor, tissue, and promoter specificity of coactivators. As this knowledge evolves, the ability to inhibit these interactions specifically will pave the road to novel and more refined therapies for hormone-dependent diseases.

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Selected Reading

1. Geistlinger, T.R., and Guy, R.K. (2003). J. Am. Chem. Soc. *125*, 6852–6853.
2. Aranda, A., and Pascual, A. (2001). Physiol. Rev. *81*, 1269–1304.
3. McKenna, N.J., and O'Malley, B. (2002). Cell *184*, 465–474.
4. Heery, D.M., Kalkhoven, E., Hoare, S., and Parker, M.G. (1997). Nature *387*, 733–736.
5. Renaud, J.P., and Moras, D. (2000). Cell. Mol. Life Sci. *57*, 1748–1769.
6. Nolte, R.T., Wisely, G.B., Westin, S., Cobb, J.E., Lambert, M.H., Kurokawa, R., Rosenfeld, M.G., Willson, T.M., Glass, C.K., and Milburn, M.V. (1998). Nature *395*, 137–143.
7. Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A., and Greene, G.L. (1998). Cell *95*, 927–937.
8. Darimont, B.D., Wagner, R.L., Apriletti, J.W., Stallcup, M.R., Kushner, P.J., Baxter, J.D., Fletterick, R.J., and Yamamoto, K.R. (1998). Genes Dev. *12*, 3343–3356.
9. McInerney, E.M., Rose, D.W., Flynn, S.E., Westin, S., Mullen, T.M., Krones, A., Inostroza, J., Torchia, J., Nolte, R.T., Assa-Munt, N., et al. (1998). Genes Dev. *12*, 3357–3368.
10. Chang, C.-Y., Norris, J.D., Gron, H., Paige, L.A., Hamilton, P.T., Kenan, D.J., Fowlkes, D., and McDonnell, D. (1999). Mol. Cell. Biol. *19*, 8226–8239.
11. Northrop, J.P., Nguyen, D., Piplani, S., Olivan, S.E., Kwan, S.T.-S., Go, N.F., Hart, C.P., and Schatz, P.J. (2000). Mol. Endocrinol. *14*, 605–622.
12. Qi, C., Zhu, Y., Pan, J., Yeldandi, A.V., Rao, M.S., Maeda, N., Subbarao, V., Pulikuri, S., Hashimoto, T., and Reddy, J.K. (1999). Proc. Natl. Acad. Sci. USA *96*, 1585–1590.
13. Li, X., Wong, J., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (2003). Mol. Cell. Biol. *23*, 3763–3773.
14. Sadow, P.M., Koo, E., Chassande, O., Gauthier, K., Samarut, J., O'Malley, B.W., Seo, H., Murata, Y., and Weiss, R.E. (2003). Mol. Endocrinol. *17*, 882–894.
15. Gehin, M., Mark, M., Dennefeld, C., Dierich, A., Gronemeyer, H., and Chambon, P. (2002). Mol. Cell. Biol. *22*, 5923–5937.
16. Hong, H., Darimont, B.D., Lang, L., Yamamoto, K.R., and Stallcup, M.R. (1999). J. Biol. Chem. *274*, 3496–3502.
17. Tremblay, A., Tremblay, G.B., Labrie, F., and Giguere, V. (1999). Mol. Cell *3*, 513–519.
18. Cheskis, B.J., McKenna, N.J., Wong, C.-W., Wong, J., Komm, B., Lyttle, C.R., and O'Malley, B.W. (2003). J. Biol. Chem. *278*, 13271–13277.

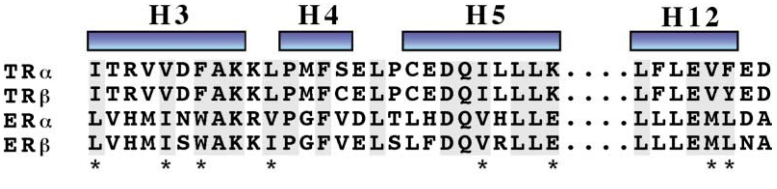

Figure 2. The Hydrophobic Groove of Nuclear Receptors Is Highly Conserved

The hydrophobic groove in the nuclear receptor ligand binding binding domain is formed by residues of α helices 3 (H3), 4 (H4), 5 (H5), and 12 (H12) [6–8]. Shown is an alignment of the corresponding sequences of the human thyroid hormone receptors (hTRα, hTRβ) and estrogen receptors (hERα, hERβ). Residues that form the hydrophobic groove are shaded. Groove residues that are variant are labeled with an asterisk. Most replacements of these residues are conservative.