Point of VIEW

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Chemical Genomics: Dialed in Transcriptional Network Control with Non-steroidal Glucocorticoid Receptor Modulators

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ABSTRACT A recent study analyzed the transcriptional effects induced by a panel of non-steroidal glucocorticoid receptor modulators. The authors discover patterns of cell-, gene-, and mechanism-specific regulation, with implications for development of improved anti-inflammatory agents.

Nuclear hormone receptors (NHRs) act as transmitters to communicate the hormonal status of an organism to the nucleus where they initiate and modulate transcriptional programs (*1*). Nuclear receptors are attractive drug targets because they have the power to control transcription at several gene loci, thereby having powerful and lasting effects on cellular processes. However, targeting the combinatorial nature of gene transcriptional regulation by NHRs also has drawbacks because undesirable transcriptional reprogramming leads to serious side effects. Therefore, a major challenge in NHR drug discovery is achievement of gene-specific regulation. For example, selective estrogen receptor modulators (SERMs) (*2*) are able to decouple some of the functions of the estrogen receptor. A parallel venture is development of selective glucocorticoid receptor modulators (SGRMs) (*3*) that retain the desired anti-inflammatory and immunosuppressive functions of glucocorticoids but do not induce harmful side effects such as osteoporosis (bone loss), and metabolic disorders. In the March issue of *Genes and Development* (*4*), a new class of non-steroidal arylpyrazole compounds, designed by Shah and Scanlan (*5*), were shown by Keith Yamamoto's laboratory to modulate glucocorticoid receptor (GR)-mediated transcription in a gene- and cell-specific manner. Although the panel of compounds all bind GR with nanomolar affinities and differ from each other by substitution at a single position, the transcriptional profiles and resultant cellular effects varied dramatically. Many of the compounds were able to induce the desired anti-inflammatory effects of glucocorticoids without affecting osteoblast (bone cell) differentiation, potentially decoupling some of the beneficial effects of GR activation from detrimental effects such as osteoporosis. Additionally, such compounds are valuable tools to study how subtle changes in ligand structure affect GR conformation and the resulting biological output.

GR is a ligand-inducible transcriptional regulator, comprising DNA, ligand, and protein–protein interaction domains. Upon ligand binding, inhibitory chaperones are shed and the receptor enters the nucleus, where transcriptional regulation occurs by three mechanisms: (i) directly binding to simple glucocorticoid response elements (GREs), (ii) cooperatively binding at promoters that contain GREs and additional transcription factor binding sites (composite GREs), and (iii) allosteric tethering through nonreceptor transcription factors. Transcriptional regulation by GR reflects information integrated from promoter architecture, ligand structure, and cofactor composition. It is believed that differential utilization of GR protein surfaces dictates GRE and cofactor binding (*6*). Small molecule control of regulatory surface availability is better understood in the case of the estrogen receptor, where structural studies revealed that different SERMs induce different transactivation domain conformations (*7*), affecting corepressor recruitment. The

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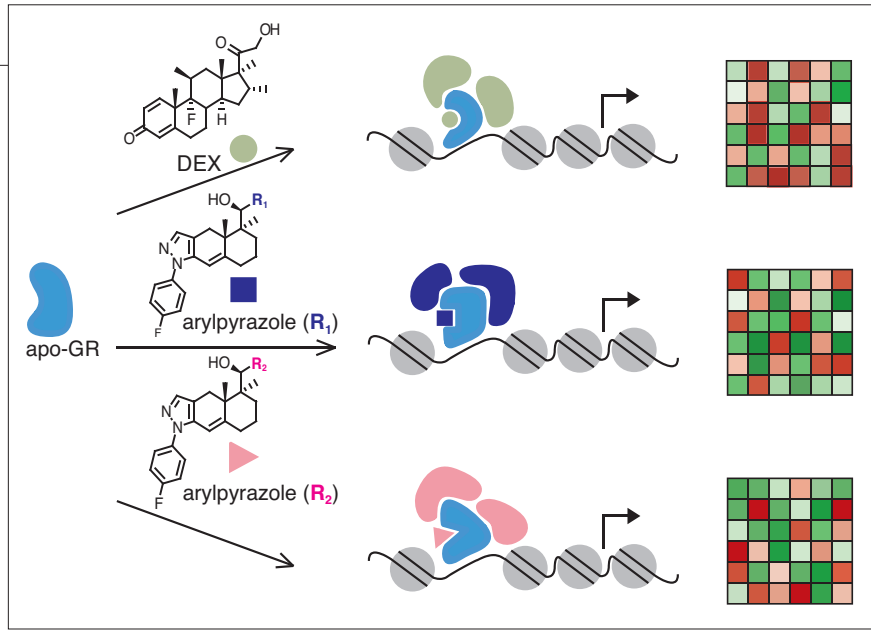

Figure 1. Model of the effects of ligand structure on GR-mediated transcription. Upon binding different ligands different GR regulatory surfaces are exposed, resulting in distinct cofactor recruitment. Extrapolating these effects to a set of GR-regulated genes yields compound-specific transcriptional signatures.

ability of ligands to modulate protein–protein and protein–DNA interactions is manifested in the activation or repression of genes that require these interactions.

What does the ideal therapeutic GR transcriptional profile look like? Unfortunately these details are not known, but it is anticipated that an ideal SGRM would influence transcription at a subset of glucocorticoid-regulated genes while leaving others unaffected. The experimental approach taken by the Yamamoto and Scanlan laboratories addressed the relationship between chemical structure and the resulting transcriptional output, by systematically correlating the arylpyrazole scaffold substituents with expression profiles. First, they established the effects of the compounds in cell-based assays. Like dexamethasone (DEX), a classical synthetic glucocorticoid, many of the compounds were able to suppress growth of glucocorticoid target cells that respond to pro-inflammatory signals. A different set of the compounds induced differentiation of pre-adipocytes, suggesting that subsets of compounds affect transcription by distinct mechanisms. Most provocatively, none of the compounds inhibited differentiation of pre-osteoblasts, which is strongly inhibited by DEX.

All but one of the compounds activated a GRE containing reporter plasmid, but when the authors profiled the expression of 17 endogenous GR-regulated genes, distinct patterns of activation and repression were observed with each compound, highlighting the importance of chromosomal context. Differences in expression were then correlated with GR occupancy; some of the compounds inhibited or enhanced GR binding to GREs, but others had modest effects. One compound in particular allowed GR to bind the epithelial sodium channel GRE, but transcription was not activated. This was correlated with a lack of histone acetylation, an activating chromatin modification. Presumably, this compound rendered GR unable to recruit a histone acetlyase. At another promoter this same compound inhibited the GR:GRE interaction. Revealing promoter-specific requirements and exemplifying how mechanistic questions can be addressed with this panel of chemical tools.

Chemical genomics, the study of small molecule regulation of gene expression, is being advanced by both the development of techniques to rapidly analyze transcription networks and the availability of appropriate chemical tools. Through fruitful collaborations between synthetic chemists and biologists who study the fundamentals of transcription, we are beginning to understand how subtle chemical changes to GR ligands lead to biological effects mediated through genetic reprogramming. Whether these arylpyrazoles or similar compounds will become pharmaceutical agents remains to be seen, but their utility as chemical tools for systematically studying mechanisms of GR-mediated transcription is already clear.

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