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Previews

Augmenting Vitamin D to Combat Genetic Disease

Vitamin D-resistant rickets is a genetic disease that causes severe bone underdevelopment due to mutations in the vitamin D receptor. Orthogonal analogs of vitamin D were recently identified that correct defects in the ligand binding pocket of a mutant receptor associated with this disease.

Vitamin D is a small molecule hormone that regulates numerous physiological processes, including calcium and phosphate absorption and bone development, by binding to membrane-associated and nuclear vitamin D receptors (VDRs). The nuclear VDRs control gene expression by heterodimerizing with retinoid X receptors (RXR). Vitamin D promotes this protein-protein interaction and initiates a complex series of molecular events, including recognition of specific DNA sites by the receptor complex, recruitment of coactivator proteins to DNA, and regulation of gene expression involved in the proliferation and differentiation of bone-forming osteoblast cells. Insufficient vitamin D results in rickets, a disease characterized by softening and weakening of bones. Although most cases of rickets can be prevented or treated with vitamin D or related VDR agonists, individuals with the genetic disease vitamin-D-resistant rickets (VDRR) do not respond to this treatment. Patients with this rare but devastating genetic disease experience severe bone underdevelopment. This is a result of mutations within the VDR, some of which directly block binding of vitamin D to its receptor.

Previous work by a number of laboratories has provided a chemical genetic strategy for complementing these types of mutations using synthetic small molecules [1, 2]. This strategy has roots in the field of genetics, where protein functions lost by mutation can often be rescued by compensatory mutations (also known as second-site revertants) [3]. The related chemical genetic approach can be employed to identify synthetic ligands that are complementary to mutant proteins and rescue loss-of-function mutations. This approach has enabled the identification of orthogonal ligands complementary to numerous mutant proteins, including the immunophilins [4, 5], estrogen receptors [6–8], RXR [9], p53 [10], and protein kinases [1].

Professor John Koh and his group at the University of Delaware recently reported [11, 12] a chemical genetic approach for the treatment of VDRR. Using the X-ray crystal structure of the VDR bound to the biologically active vitamin D metabolite $1,25(OH)_2D_3$ [13], the Koh laboratory constructed a computational model of a key arginine to leucine (R274L) mutant known to cause VDRR by directly interfering with vitamin D binding. The energy of this hydrophobic leucine side chain in the ligand binding pocket of the model receptor was minimized with a conformational search algorithm. Modeling indicated that this mutation results in loss of a critical hydrogen bond between the 1-hydroxyl group of vitamin D and opens a hydrophobic "hole" adjacent to the ligand. Potential candidate compounds were designed to fill this hydrophobic hole based on modified structures of vitamin D and the synthetic agonist L190155 (see figure). Hydrophobic groups appended to these compounds were envisioned to confer additional hydrophobic interactions within the ligand binding pocket and enable binding and proper activation of this defective receptor. These compounds were subjected to a virtual screen against the VDR R274L mutant model using Monte Carlo docking simulations to identify those compound candidates with the highest predicted association energies. Using this rational design approach in conjunction with small focused libraries of compounds, the Koh laboratory synthesized and evaluated vitamin D analogs derived from $1,25(OH)_2D_3$ and L190155.

The rational design strategy employed by the Koh laboratory successfully identified novel compounds that activate the R274L mutant VDR with up to 500-fold greater potency than existing VDR agonists. Importantly, these compounds were selective for this nuclear VDR and did not stimulate the activity of a VDR that acts at the cellular plasma membrane. Compounds that rescue only the defective nuclear VDR and do not activate the membrane VDR were desired because this membrane receptor is responsible for rapid and diverse nongenomic effects of vitamin D [14]. Moreover, aberrant activation of signaling pathways controlled by the membrane VDR is thought to preclude the clinical use of high doses of $1,25(OH)_2D_3$ as a treatment for VDRR. In contrast to the natural hormone, analogs of vitamin D were previously suggested as potential therapeutics for VDRR by Dr. Sara Peleg of the M.D. Anderson Cancer Center, who investigated a $1,25(OH)_2D_3$ derivative bearing a hydroxymethyl substituent in place of the more polar 1-hydroxyl group. The Peleg laboratory reported that this vitamin D analog at least partially rescued activation of gene expression by the defective R274L VDR mutant [15].

The results of Koh and coworkers provide additional evidence at the molecular level that certain genetic diseases might be corrected with appropriately designed small molecules. These results suggest that individuals with VDRR could be profiled by genetic screening to identify mutations capable of complementation by small molecules. Although the number of potential candidates will be limited by the large number of mutations known to cause VDRR and the low frequency of mutations that form direct contacts with vitamin D ($\sim$2 out of 20 known mutations [16]), this general rational design approach holds promise for the rapid identification of potent lead compounds that rescue functions of specific mutant members of the nuclear hormone receptor superfamily.

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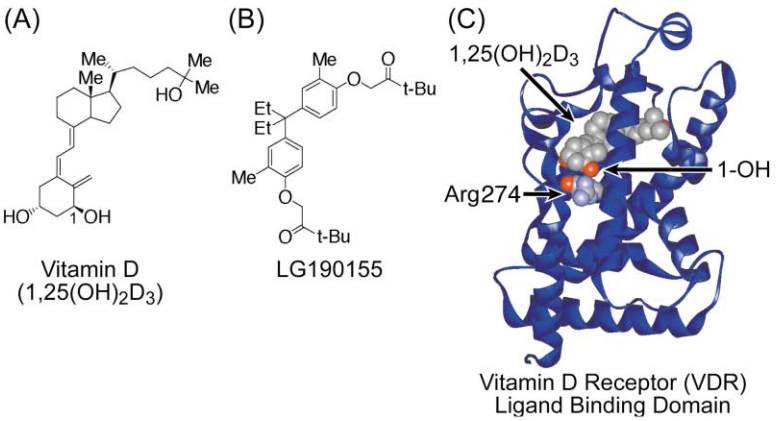

Natural and Nonnatural Vitamin Ds Compared with the Protein-Bound Vitamin

Structures of the active vitamin D metabolite $1,25(OH)_2D_3$ (A), the related synthetic agonist LG190155 (B), and the $1,25(OH)_2D_3$-VDR complex (C). In (C), the VDR arginine residue (Arg274) known to be mutated to a smaller hydrophobic leucine residue in cases of vitamin D-resistant rickets and the proximal 1-hydroxyl group (1-OH) of $1,25(OH)_2D_3$ modified to compensate for this mutation are explicitly shown.

Selected Reading

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Control in the N-linked Glycoprotein Biosynthesis Pathway

N-linked glycosylation is vital for the development and maintenance of eukaryotic cells. The individual steps of this complex process are slowly being elucidated. In this issue, the Imperiali group further dissects the mechanics of oligosaccharyl transferase using substrate analogs.

The glycosylation of proteins requires a significant commitment of genetic and energetic resources by eukaryotic cells. Since this complex process was discovered, researchers have been trying to uncover and understand the various steps and molecular mechanisms of glycosylation that nature accomplishes with apparent ease. The critical importance of N-linked (or asparagine-linked) glycosylation to multicellular organization was demonstrated several years ago by Lennarz, who treated sea urchin embryos with tunicamycin to completely ablate N-linked glycosylation and observed the cessation of embryonic development [1]. Since that time, advances in gene manipulation have allowed researchers to conduct more elegant experiments to dissect these processes at the molecular level, and ultimately lend support to the hypotheses generated by Lennarz's earlier work. Marth and colleagues strived to disrupt the key glycosyltransferase genes using homologous recombination in mouse, and in this way showed that deletion of these genes resulted in embryonic lethality; interestingly, the earlier in the pathway the gene is deleted, the less progressive the embryonic development. Specifically, in this study, Marth and coworkers demonstrated that disrupting the gene that encodes for the dolichol GlcNAc-1-phosphotransferase (see figure), the enzyme inhibited by tunicamycin, blocks mouse development at gastrulation, precisely as Lennarz had shown earlier in sea urchins [2, 3]. By contrast, mouse embryos that were homozygous for a disrupted gene encoding the protein N-acetylglucosylaminyltransferase I, an enzyme that acts at a later stage in the glycosylation pathway for the synthesis of complex N-linked oligosaccharides, progress through the initial developmental events, but stop abruptly at embryonic stage E9.5–E10.5 [4, 5].

Intervening in the early steps in the dolichol lipid pathway for N-linked glycosylation by either chemical or