Spring 2017

Structural Basis for Mutant RXRA-Mediated Hyperactivity of PPARs in Bladder Cancer

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Understanding the structural mechanism by which an oncogenic point mutation promotes aberrant activity is prerequisite to rational drug design. Recent genomic characterization of bladder tumors has suggested several oncogenes relied upon for tumor development and thus ideal for targeted inhibition. The nuclear receptor RXRA contains a hotspot mutation and posits one such candidate oncogene. Growth assays performed by our group demonstrated mutant RXRA drives proliferation in a bladder urothelial organoid model, confirming the recurrent mutation's oncogenic potential. RXRA activates transcription as a homodimer or obligate heterodimer with sixteen other nuclear receptors. The hotspot mutation is located at the heterodimerization interface and contacts binding partners. Co-overexpression of RXRA with known heterodimerization partners revealed mutant-mediated hyperactivity occurred solely when in complex with the PPAR class of nuclear receptors, suggesting mutant RXRA is oncogenic in a narrow range of structural contexts. Based on this structural specificity, I speculate the RXRA/PPAR complex represents a targetable oncogenic dyad.

To define the mechanism of mutant RXRA-mediated hyperactivation of PPARG, the PPARG ligand-binding pocket and transactivation helix (responsible for transcriptional machinery recruitment) were mutated. Hyperactivity occurred independent of ligand binding but relied on the PPARG transactivation helix, suggesting an allosteric relay induces PPARG into an active conformation. To identify the residues comprising the allosteric relay, long-timescale molecular simulations and subsequent Markov state modeling of RXRA/PPARG were performed. Predicted metastable states for the mutant heterodimer illustrate the PPARG transactivation helix to predominantly adopt conformations similar to the agonist-bound crystal structure, in contrast to conformations adopted by the wild-type dimer. The PPARG terminal tyrosine on the mutant heterodimer occupied a distinct region in space compared to the wild-type heterodimer, suggesting a functional relevance for this residue. Elimination of the PPARG terminal tyrosine prevented mutant-mediated hyperactivity. These insights will guide future chemistry efforts to inhibit.