論文の内容の要旨

論文題目

Towards specific inhibition of SIRT2 deacetylase by a macrocyclic peptide inducing dynamic structural change

(環状ペプチドにより惹起されるサーチュイン 2 の構造変化、及び特異的阻害剤に向けて)

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SIRT2 deacetylates specific acetyllysine residues in diverse proteins, and is implicated in a variety of cellular processes. The seven mammalian sirtuins, SIRT1–SIRT7, have distinct substrate specificities and subcellular localizations, and are implicated in a variety of biological processes, such as gene silencing, cell-cycle regulation, metabolism, apoptosis, longevity and cancer. SIRT2 is primarily localized in the cytoplasm and nucleus, and it deacetylates specific acetyllysine residues in diverse proteins. For instance, the SIRT2-catalyzed deacetylation of α-tubulin and histone H4 functions in the regulation of cell-cycle progression, and its deacetylation of receptor-interacting protein 1 (RIP1) is responsible for the modulation of RIP1–RIP3 complex formation, and thus regulates programmed necrosis. In addition, the siRNA-mediated down-regulation of SIRT2 induces apoptosis in HeLa cells, caused by p53 accumulation. Moreover, SIRT2 is associated with the aggregation of neural proteins such as α-synuclein and huntingtin, and thus SIRT2 inhibition prevents neurodegeneration in Parkinson’s disease and Huntington’s disease models. Collectively, the specific inhibition of SIRT2 has tremendous potentials to treat various human diseases

We have recently developed a series of ε-trifluoroacetyllysine-containing macrocyclic peptides by means of RAPID system, which inhibit the SIRT2 activity more potently than most other known inhibitors. In spite of these facts, it remains unclear how these macrocyclic peptides strongly bind SIRT2 and exhibit their inhibitory activities. Here we report the crystal structure of human SIRT2 in complex with a macrocyclic peptide inhibitor, S2iL5, at 2.5-Å resolution.

The structure revealed that S2iL5 binds to the active site of SIRT2 through extensive interactions. S2iL5 binds to the active-site groove between the small and large domains of SIRT2, with the $K^{Tfa}7$ side chain inserted into the catalytic tunnel formed by His187, Val233 and Phe235. The hydrocarbon chain of $K^{Tfa}7$ is sandwiched by the side chains of His187 and Phe235, with its Nε atom hydrogen bonded to the carbonyl group of Val233.

A comparison between the SIRT2 structures in complex with S2iL5 and in the free form revealed the conformational changes that occur in SIRT2 upon S2iL5 binding. First, the small domain moves toward the large domain upon S2iL5 binding, as observed in other sirtuins. This open-to-closed domain movement allows Phe235 to form the catalytic tunnel, and enables Gly236, Glu237 and Gln267 to form the β sheet-like interactions with S2iL5. Second, the SIRT2-specific insertion (residues 289–304) adopts a loop conformation to interact with S2iL5 in the S2iL5 complex, whereas it forms an α-helix in the free form. The side chain of Asp294 hydrogen bonds with the amide groups of Phe296 and Gly302, to stabilize the loop conformation. Third, S2iL5 binding induces a local conformational change, including the rotation of the side chains of Phe243 and Phe244, which are involved in the interactions with S2iL5 Our findings unveiled the potential of macrocyclic peptides to bind target proteins by inducing dynamic structural changes.

To confirm the functional significance of the observed interactions between SIRT2 and S2iL5, we determined the K_D values between S2iL5 and alanine mutants of SIRT2, by surface plasmon resonance (SPR). The F244A mutant showed the most pronounced reduction in the affinity for S2iL5, confirming the importance of the interactions between Phe244 and S2iL5. The E116A and E120A mutants showed reduced affinity for S2iL5, establishing the importance of the salt bridges between R9 of S2iL5 and Glu116 and Glu120 of SIRT2. We also determined the K_D values between SIRT2 and alanine mutants of S2iL5. Among the S2iL5 mutants tested, the R9A mutant showed the most pronounced reduction in the affinity for SIRT2, confirming the importance of the salt bridges between R9 of S2iL5 and Glu116 and Glu120 of SIRT2. The Y4A/R9A/Y12A triple mutant showed an additive decrease in the affinity as compared with the single mutants (Y4A, R9A and Y12A) and the double mutants (Y4A/R9A, R9A/Y12A and Y4A/Y12A). Notably, the Y4A/R9A/Y12A mutant showed markedly reduced affinity as compared with the Y4A/R9A mutant, although the Y12A mutant showed no reduction in the affinity. These results indicated that the multiple interactions synergistically contribute to the binding of S2iL5 to SIRT2. We further determined the K_D values between SIRT2 and a 14-aa linear peptide, in which the amino group of Y1 in S2iL5 is acetylated, and a 5-aa short linear peptide corresponding to H5–R9 of S2iL5. The 14-aa linear peptide showed a marked decrease in the affinity (K_D = 6.8 nM), whereas the 5-aa linear peptide showed a drastic reduction in the affinity (K_D = 145 nM). Altogether, our mutational analyses confirmed the functional significance of the observed binding mode between SIRT2 and S2iL5, and indicated the synergistic contributions of the multiple interactions.

From these results, the present structure of SIRT2 in complex with S2iL5 revealed that S2iL5 binding induces a conformational change in the SIRT2-specific region. Our functional data demonstrated the potential role of this dynamic structural change for S2iL5 binding. It seems

unlikely that small molecule inhibitors could induce such large structural changes in their target proteins, due to the limited number of inhibitor–protein interactions. Thus, the ability to bind to a target protein, by inducing a structural change within the target, is likely to be a unique advantage for macrocyclic peptide inhibitors.