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Rational Drug Discovery for Inhibiting Ubiquitination of Magnesium Channels

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Summary

We are focusing on the paracellular Mg^{2+}-selective channel that is responsible for reuptake of Mg^{2+}. The paracellular Mg^{2+} is composed by two claudins (CLDNs), CLDN16 and 19, the molecules involved in intercellular adhesion. It is known that the physiological stability of these CLDNs is controlled by LNX3, a specific E3 ubiquitinating enzyme. In this study, we tried to discover small molecules that inhibit the interaction between LNX3 and CLDN16/19, since such molecules could lead to the development of LNX3 inhibitors. Transient inhibition of degradation of CLDN16/19 by LNX3 may result in recovering disfunction of magnesium homeostasis, thereby offering a new crew for development of novel drugs for hypomagnesemia. In vitro binding experiments with the bacterially expressed proteins showed that the first PDZ domain of LNX3 (LNX3-PDZ1) bound the C-terminus of CLDN16. We further confirmed CLDN3 and CLDN19 did not show any binding. On the other hand, between the second PDZ domain (LNX3-PDZ2) and the C-termini of both CLDN16/19 were unclear and seemed non-specific, although certain interaction was observed.

Next, the authors succeeded in discovering four non-peptidic inhibitors against LNX3-PDZ1 by using the NMR-screening method. This screening was performed using our original focused library of compounds of anthranilic acid derivatives, since we already identified that anthranilic acid is the common pharmacophore for pan-PDZ domain specific inhibitors. Unfortunately, all these prototype compounds did not show enough high affinity against LNX3-PDZ1. It is unlikely to become a lead compound for further drug development. Then, we attempted to advance the precise molecular design of LNX3-PDZ1 specific inhibitors with structural information of atomic resolution by X-ray crystallography of LNX3-PDZ1 / CLDN16 complex. At present, we have succeeded in obtaining a crystal that gives diffractions whose resolution is equivalent to approximately 3.5 Å. Further structural refinement is now on the way.