Structural insight into the neosubstrate selectivity of thalidomide metabolite

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Thalidomide (Thal) exerts adverse effects such as teratogenicity, however, is used for the therapy of multiple myeloma and other haematologic malignancies as immunomodulatory imide drugs (IMiDs). The molecular mechanism of thalidomide's pharmacological action has been gradually elucidated through the search for multiple target proteins that thalidomide acts on. Celebron (CRBN) is the intracellular receptor for Thal and induces Thal-dependent degradation of target protein (neosubstrate) as a component of a E3-ubiquitin ligase. Although C2H2 zinc finger (ZF) transcription factors, IKZF1 and SALL4, are concerned in immunomodulatory effects and teratogenicity of Thal, respectively, a primary Thal metabolite, 5-hydroxythalidomide (5HT), induces degradation of SALL4 but not IKZF1. Due to the action of the enzyme cytochrome P450 in the body, the administered thalidomide produces 5HT. Here, we focused on the molecular mechanism in which the selectivity of Thal toward C2H2 ZF-type neosubstrates is altered with its metabolism. First, we characterized the enantioselectivity of the formation in the SALL4-CRBN complex. The (S)-enantiomer of Thal and 5-HT showed more effect than the (R)-enantiomer, which is consistent to "Left-hand (S-form) theory of teratogenicity" of Thal. Based on the enantioselectivity, we determined the crystal structures of the ternary complexes of the Thal-binding domain (TBD) of human CRBN and the second ZF domain (ZF2) of human SALL4 induced by (S)-Thal and (S)-5HT. As a result, Thal and 5HT positioned between the interface of SALL4 ZF2 and CRBN TBD to mediate the protein-protein interaction as molecular glues. Although both compounds occupy at the same position in the SALL4-CRBN complex, the 5-hydroxy group of 5HT forms an additional hydrogen bond with CRBN TBD through a water molecule, which enhances the formation of the SALL4-CRBN complex. The 5-hydroxy group is also located near the 2nd and 9th residues of the β-hairpin structure in SALL4 ZF2, and these residues are different from IKZF1. The complex formation and proteasomal degradation experiments using the residue-swap mutants of SALL4 and IKZF1 elucidated the variation in the 2nd residue of β-hairpin structure defines the neosubstrate selectivity of 5HT. Thalidomide's action on its target is altered through its metabolism in the body and if the hydroxylation of thalidomide found in this study is avoided, a new designed drug can be expected to reduce teratogenicity. Furthermore, our findings indicate that the structural differences found in C2H2 ZF-type transcription factors may be exploited to increase the efficiency of action of IMiDs, including thalidomide, on target proteins required for drug efficacy.